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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES ANNUAL REPORT

FY 1977

July 1, 1976 through September 30, 1977

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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

The Institute has begun its second decade of research into those environmental factors that disrupt the biological processes within the human organism, and cause disease, disability and death.

The list of accomplishments and accumulation of scientific data within the Institute continues to grow, but what is past is prologue. It is a foundation upon which we must build to obtain even more knowledge about those pollutants being introduced at an increasing rate each and every year into our environment. If the human race is to survive upon this earth, we must be continually dilligent to arbitrate the dichotomy between commercial expediency and the well-being of our own and future generations.

From its arsenal of intellegence, the Institute has been able to select the appropriate weaponry to make determinations concerning recent attacks upon the environment. Significant among these has been the exposure to polybrominated biphenyls (PBBs) by the inhabitants and livestock in the State of Michigan.

The livestock were inadvertantly fed a mixture that contained a PBB fire retardant, instead of their regular supplement of magnesium oxide. The toxic PBBs were then passed on to the human population through meat obtained from the slaughtered animals and other dairy products. The Institute, through a Center grant, is now investigating a cohort of residents to determine if there have been any health effects associated with the ingestion of this toxic chemical. In one peculiar instance in the State a farmer whose dairy herd was affected by PBBs, recattled his farm and housed them in a new barn constructed of lumber treated with pentachlorophenol (PCP). The new herd began to show signs of illness, including respiratory disease, increased perinatal mortality, and increased birth defects. NIEHS tests on these animals found elevated amounts of toxic dioxins, a by-product of pentachlorophenol manufacture. The Institute is investigating the possible synergistic effects of PBB and PCP as well as other public health implications of this type of exposure in humans.

Akin to the PBBs is another group of toxic chemicals identified as PCBs or polychlorinated biphenyls. These substances have many industrial applications, such as in electrical transformers, and capacitors, etc. Industry is aware of the toxicity of this chemical and Congress has mandated that manufacture close. However, hundreds of millions of pounds remain in the environment.

This store of PCB is a potential threat to the general population, that feeds upon fish caught in waters polluted by the effluent from manufacturing plants along some rivers and lakes. The chemical therefore has found its way into

the human food chain. NIEHS is collaborating with other federal agencies and scientists throughout the country to learn more about the level of insult from PCBs that the body can tolerate. Research has already determined that PCBs accumulate in adipose tissue. Mothers who nurse their young are passing quantities of these PCBs to their offspring in their milk. A study is being launched in North Carolina to obtain milk from mothers at regional hospitals for evaluation of PCB content. The children will be evaluated for any developmental idiosyncracys.

Just how many and which of the nearly 1000 new chemicals introduced into the environment each year from industry are toxic to humans, or may be mutagenic, is of prime concern. A newly-initiated Mutagenesis Testing Program will look at substances, that by their chemical structure are suspect. The substances will be evaluated in a blind study using a hierarchy of tests, proceeding from rapid screening in simple, highly sensitive microbial systems, through in vitro mammalian systems, up to whole animal tests. Substances found to be mutagenic in the microbial test will be given first priority for additional testing. First priority substances will be tested in higher systems that serve to affirm or deny mutagenicity. Tests will run through three tiers, the microbial system, the Drosophila system, and the whole animal (mammalian) system. Results will be computerized with assistance from the Division of Computer Research and Technology in Bethesda and made readily available to other Federal agencies, industry, legislators, and the general public.

To further expedite the transfer of technology we have established an Environmental Teratology Information Center (ETIC) at the Oak Ridge National Laboratory a component of the Department of Energy (DOE).

This center collects reprints, enters bibliographic information on magnetic tape, and prepares extracts of publications containing data pertinent to determining the teratogenic potential of environmental agents to which man is exposed. It also lists investigators with studies in progress on given agents. The center was established in response to the need for a central location for information on what is in the literature on what specific chemicals or agents have been tested, to what extent, and on what species.

Information collected on birth defect-causing agents is now available through the National Library of Medicine's TOXLINE and Toxicology Data Bank.

In the Laboratory of Environmental Mutagenesis, investigators have developed a technique to determine whether any person or groups have been exposed to mutagens. Using data from this newly developed screening technique, investigators will be able to determine the mutation frequency in normal populations. Then persons who have been exposed to some mutagenic event can be readily identified, by an increase in this frequency. The technique is simple, antibodies that react only to mutant hemoglobins are introduced into blood samples. When these antibodies are coupled with a flourescent marker, they immediately identify the mutant cells under a fluorescent light microscope.

By counting the aberrant cells with a computer, the increased frequency of mutations can be ascertained, indicating expsoure to a mutational force. The

investigators are using the hemoglobin S molecule in their test because it is a known mutation but the whole spectrum of abnormal hemoglobins come under investigation one by one until any increase in mutation frequency is noted. The specific mutant causing the increased mutation frequency can then be identified.

The research that the Institute has accomplished over the past year is as important and comprehensive.

The scientific capability of the Institute has recently been enhanced by the addition of several prominent scientists, among them are:

Dr. Colin Chignell, Chief of the Laboratory of Environmental Biophysics, who has received the John J. Abel award from the American Society for Experimental Therapeutics for his work on the mechanism of drug-macromolecule interaction.

Dr. John W. Drake, Head of the Molecular Genetics Section in the Environmental Mutagenesis Branch, who has been a Fulbright, a Guggenheim Fellow, and an NIH Special Fellow. From 1969 until he joined the Institute, he was Professor of Microbiology at the University of Illinois at Urbana, and directed the Ph.D. program in genetics there.

Dr. Clifford Mitchell, has been hired as Chief of the Laboratory of Behavioral and Neurological Toxicology. He has been the Manager of Nervous System and Cardiopulmonary Pharmacology at the Riker Laboratories in St. Paul, Minnesota since 1974, and since 1973 has held a Lectureship in Pharmacology at the University of Minnesota.

Dr. Paul Nettesheim, an expert in pulmonary pathology and environmental carcinogenesis of the lung, is now Chief of our Pulmonary Function and Toxicology Laboratory.

These eminent scientists are among those at the Institute who will guide us in our quest to identify, and attempt to ammeliorate those substances in the environment that pose a threat to mankind.

These and the other NIEHS scientists will eventually be working in the NIEHS permanent facility now under construction on T.W. Alexander Drive, in Research Triangle Park. Work on the building is progressing on schedule.

When completed in 1980, the facility will be a unique structure comprising the latest innovations in laboratory flexibility, administrative convenience, and environmental safety and compatibility.

In the laboratories, scientists will be pursuing and examining those environmental pollutants that prey upon the well-being of man's future generations.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

Research Grants

During FY-1977, 247 Regular Research, Program Project, and Research Career Development Award applications were reviewed, a slight decrease from FY-1976. Awards were made to 102 noncompeting and 120 competing projects from FY-1977 appropriations, for a total of $18.4 million. In addition, 17 projects for a total of $1.5 million were supported from energy related pass-through funds provided under an interagency agreement with the Environmental Protection Agency.

Manpower Development Resources

Four Institutional training grants awarded prior to the passage of Public Law 93-348 received a total of $625,000. This was the terminal award for these grants in accordance with the phase-out provisions of the law. Seven Institutional National Research Service Awards initiated in 1975 were continued at a level of $919,000. The FY-1977 appropriation provides for Individual Fellowships at a level of $1.0 million and 15 new Institutional Awards for $1.5 million.

Since NIEHS is the only Federal agency currently supporting training in the area of toxicology, the NIEHS staff is concerned that current training funds available will result in a tremendous deficit in the number of qualified people trained over the next few years to protect the public health and implement the mandates of the Toxic Substances Control Act.

A summary of each of the Institutional training grants currently supported in the primary thrust areas of environmental toxicology, environmental epidemiology and biostatistics, environmental pathology and environmental mutagenesis appears in this report.

Environmental Health Sciences (EHS) Center Programs

Environmental Health Sciences Centers, which are university-based, comprise an essential national resource and are an integral part of the strategy of NIEHS in serving the national needs in environmental health research. These Centers perform high quality, multidisciplinary research in areas of high relevance to the mission of NIEHS and serve to attract and train outstanding students to meet future research needs of the Nation. During FY-1977, the NIEHS supported seven EHS Centers with funds totaling $7.2 million.

Because of the importance of the EHS Centers in meeting National needs, communication between the Centers, the Institute Director and his staff, and the National Advisory Environmental Health Sciences Council (NAEHSC) is maintained at an effective level through bi-annual meetings of the Center Directors, frequent visits and telephone contacts by extramural staff. Members of the NAEHSC Subcommittee for Environmental Health Sciences Centers frequently accompany staff on oversite visits, as well as attending Center Directors' meetings. The Subcommittee provides liaison between the Institute, the Advisory Council, and the Center Directors by keeping abreast and discussing research problems and accomplishments, new environmental concerns, and program directions.

The Fifteenth Semi-annual Meeting of the Center Directors was held on October 17-19, 1976, at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, with Dr. David P. Rall, Director, as host. In addition to NIEHS staff and Center Directors, the NIH/OD staff was represented by Dr. Ann Kaufman and Dr. Norman Mansfield. Invited speakers included Dr. Henry M. Kissman, Associate Director, Specialized Information Services, National Library of Medicine, and Dr. Sidney Wolff, Public Citizens Health Research Group, Washington, D.C. In addition to each Center Director discussing highlights of the programs being conducted in his Center, a full day was devoted to a presentation and review of the intramural scientific program of NIEHS. Since every third meeting of the Center Directors is held at the NIEHS, this further serves to keep the Center Directors aware of the main thrusts of the Institute and enables them to maintain a parallelism covering areas of need and new initiatives which the NIEHS intramural program is unable to undertake.

The Sixteenth Meeting of the EHS Center Directors was held at the University of Rochester (New York) School of Medicine and Dentistry with Dr. Thomas Clarkson serving as host. In addition to the Center Directors, the NIEHS staff and Subcommittee of the Council, Dr. Leon Golberg, President of the Chemical Industry Institute of Toxicology (CIIT) served as the invited speaker and made a most interesting presentation regarding the CIIT, its programs and objectives including the current status of development. This was of special interest to all attending in that the CIIT is funded by a consortium of companies in the chemical industry as a "public" resource for research on industrial chemicals toxicity testing and for training which has a direct relationship with many of the areas of current research at NIEHS and in the EHS Centers. Consistent with the usual format, the program at the University of Rochester was presented and reviewed for a full day with the Director, NIEHS, and other Center Directors presenting highlights of their programs on the second day.

At the request of the Senate Appropriations Subcommittee, NIEHS was requested to prepare for the spring hearings a plan for developing Marine and Freshwater Biomedical Centers. Dr. Rall made the presentation during the hearings, outlining the probable scope of such a program which would envision funding a few of the currently excellent marine institutes on a competitive basis to encourage the use of marine and freshwater species as models for environmental health research. This has previously been done on a small scale through project grants and has proved very useful in determining pollutant

levels, toxic substances, as well as physiological studies where these species present a better research model than mammalian species to study human health effects. The NIEHS is ready to mount such a program in the event appropriations are made for this type research.

Supported Research Highlights

During the year, NIEHS grantees have made substantive contributions to each of the categorical programs of the Institute. Considerable progress has been made and the understanding of basic toxicological and pathological events relating to human health, genetic and reproductive anomalies, neurological disorders and various other syndromes ascribable to environmental factors. Highlights from the programs supported during the year which illustrate these achievements are presented herewith. Of particular interest is the initiation during the year of a new research program, the Young Environmental Scientist Health Research Grant program, which was designed to encourage young investigators to develop an interest in problems of environmental health sciences and to provide support for their initial independent research efforts.

TRAINING PROGRAMS

During the past year, eleven extant institutional training grants were continued and five new institutional awards were made. The majority of these awards emphasize training in environmental toxicology. A description of the training content of this and other NIEHS emphasis areas follows:

1. Environmental Toxicology: Trainees in this area receive didactic and research training in pharmacological and toxicological principles which determine the effects of exposure to environmental agents. Pharmacokinetic and pharmacodynamic factors, cellular and molecular mechanisms of action, synergism, species variability in toxic response, and test development, design and interpretation are all facets of this training area. Graduates are qualified to pursue careers in experimental and/or predictive toxicology in academic, industry or government.

2. Environmental Epidemiology and Biostatistics: Trainees in epidemiology learn to utilize statistical and mathematical tools to assist in the identification of environmental disease in human populations. Training stresses non-infectious disease epidemiology with an emphasis on the identification of causes of environmental disease. Biostatistics trainees focus on learning and applying mathematical and statistical tools to assist environmental health scientists in experimental design and interpretation. These programs provide research epidemiologists and statisticians to analyze data from human or animal populations to determine the potential human hazard of exposure to environmental agents.

3. Environmental Pathology: Trainees in this area focus their research on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees hold professional or academic degrees which qualify them for advanced training in gross and histopathological research dealing with the structural and functional alterations of tissues exposed to environmental chemicals. Graduates are qualified to become members of research teams involved in chemical risk evaluation using experimental models, conducting the pathological phases of the research and assisting in experimental design and interpretation.

4. Environmental mutagenesis, teratogenesis and carcinogenesis: Trainees in these areas learn to apply the basic principles of genetics, embryology, and biochemistry to applied studies aimed at assessing the potential genetic or reproductive hazards to man of environmental chemicals. Carcinogenesis

training similarly is applied in nature, focusing on the assessment of environmental hazards and specific mechanisms of action rather than on basic studies of the carcinogenesis process. (This type of research training is sponsored by the National Cancer Institute.) Graduates enter careers in research aimed at understanding hazards and developing predictive tests in these fields.

Grants for Institutional support of training programs in environmental health were made to the following institutions during the past year.

The Johns Hopkins University received a grant for training in environmental toxicology emphasizing investigations of the cellular and subcellular mechanisms of action of toxic agents. This type of research training encompasses basic sciences such as biochemistry, pharmacology and physiology and trains individuals to conduct research on assay development for early signs of toxicities and on appropriate treatment methods for exposed individuals.

A broad predoctoral program in environmental toxicology was supported at Purdue University emphasizing chemical and radiation toxicology. The Schools of Pharmacy and Veterinary Medicine combine efforts to train toxicologists with research experience on the effects of exposure to heavy metals and other environmental contaminants. The University of Cincinnati training program in toxicology also received grant support for its emphasis on research training in biochemical toxicology, metabolism and safety evaluation.

A small postdoctoral training program for engineers at the California Institute of Technology is in its last year of support. The program has prepared engineers to utilize their skills for the solution of environmental health problems. Major thrusts are air and water pollution analyses. Similarly, large scale analysis air and water for pathogenic substances comprises the major thrust of a training program at the Florida State University in a joint program run by the departments of oceanography and statistics with grant support by the NIEHS.

A grant for training in environmental toxicology to the North Carolina State University emphasizes research and course work in the molecular biology and biochemistry of pesticides and herbicides. Pre- and postdoctoral training focuses on determining the effects of trace amounts of contaminants on living organisms. The training program supported at the University of California, Davis combines basic training in agricultural chemistry, biochemistry, microbiology or plant physiology with toxicology training offered by their Department of Environmental Toxicology.

At Cornell University, the NIEHS-supported toxicology program trains individuals for research in neurophysiology, neuropharmacology, mechanisms of action of pesticides, industrial pollutants and natural products. Methods for determining the metabolism and environmental fates of these agents are stressed. Postdoctoral training at the University of Wisconsin Center for

Environmental Toxicology stresses studies on pesticides, industrial pollutants and food contaminants. Similarly, the University of Illinois Institute of Environmental Studies trains students for careers in research on the pharmacokinetics and comparative toxicology of environmental substances. The movement of pollutants through ecosystems and biocide design are additional areas of emphasis at this institution.

A broad knowledge of the basic toxicological sciences is provided to students via a grant to the Institute for Experimental Pathology at the Albany Medical College. Training in histology, neuroanatomy, biochemistry, pathology, physiology, microbiology and pharmacology is applied by students to problems in environmental toxicology. Emphasis is on comparative toxicology, utilizing a variety of species and in vitro biological systems to predict toxic efforts. The Medical School of Wisconsin approaches training in toxicology by stressing pharmacological principles and observations of acute pharmacological phenomena. With this background, trainees then pursue research problems in chronic toxicity, safety evaluation and human toxicology. The University of Mississippi also has NIEHS support for a toxicology training program in a clinical environment emphasizing research on pesticides and food contaminants.

With support from the NIEHS, environmental pathobiology trainees at Washington University (St. Louis) and at the University of California, Davis study the macro- and the histopathological effects of exposure to environmental chemicals. At the University of North Carolina, the environmental pathology training program stresses etiologic and pathogenic analyses of chronic degenerative diseases.

Training in the identification of toxic materials in food and water is supported at the Massachusetts Institute of Technology where research and teaching programs emphasize the quantification and mechanisms of action of these toxicants. Natural food contaminants, pesticides and industrial pollutants all receive special emphasis in the Vanderbilt University training program with its primary thrust on resultant biochemical alterations.

Environmental epidemiology and biostatistics programs at the University of North Carolina and Yale University focus on the identification of noninfectious environmental hazards by observing samples of human populations. Other trainees learn to utilize statistical methodologies to assist in toxicological studies or to estimate risk to humans of exposure to chemicals based on data from animal tests.

These institutional training programs currently support about 175 trainees. Over 40 percent of these students are at the postdoctoral level. In addition, individual postdoctoral awards supported twenty individuals at eighteen institutions. Fifteen of these individuals were receiving research training in environmental toxicology and the remainder were receiving training in epidemiology and biostatistics.

RESEARCH HIGHLIGHTS--SPECIAL PROGRAMS

Introduction

Last year, research highlights of the Extramural Program were presented in a matrix format dealing with categories of harmful environmental factors and their effects and mechanisms. The format appeared to provide easy access to information; therefore, it has been retained in essence this year.

The broad categories of hazardous factors dealt with are:

- o Gaseous Air Pollutants
- o Suspended Particulates (Aerosols)
- o Industrial Products, Byproducts and Intermediates
- o Heavy Metals
- o Pesticides
- o Physical Factors

Gaseous Air Pollutants

Epidemiological Studies

Over the past five years, a number populations have been studied for evidence of environmentally-related lung diseases. The goal is to develop long-term dose-response data for exposures and health effects.

Firefighters. The prevalence of chronic non-specific respiratory disease was measured in 1,768 firefighters. On the basis of simple pulmonary function tests, forced vital capacity and one-second forced expiratory volume, a standardized respiratory volume, and a standardized respiratory symptom questionnaire, firefighters were classified into several categories of chronic non-specific respiratory disease. Relevant exposure information was also obtained.

Pulmonary function was measured either before and after the first shift of the week or before and after an exposure of interest. This approach has been particularly useful with irritant gases in determining whether an acute reaction occurs and, if so, whether a dose-response relationship exists. These studies frequently have the advantage of accurate exposure assessment. They also form the basis for comparison of longitudinal changes.

Experienced firefighters had a higher chronic non-specific respiratory disease rate than new firefighters of the same age group, although cigarette smoking partially obscured the occupational effect. Several indicators of occupational exposures and their resulting symptoms were associated with higher chronic non-specific respiratory disease rates.

Significantly higher disease rates were found in firefighters who smoked or had smoked cigarettes in the past. As expected, cigarette smoking was associated with a lower forced vital capacity and forced expiratory volume in one second. The latter volume was larger in ex-cigarette smokers than in current smokers, but was less than that of the never-smoker.

The majority of firefighters experienced increased mucus production and a general malaise after such exposures. Requiring oxygen at fires, being overcome, and smoke inhalation were reported with lesser but still significant frequency.

Differences in pulmonary function were noted between men of Italian and Irish origin. Smoking habits between the groups did not account for the differences. Body build and chest size might have been contributing factors.

Toluene Diisocyanate (TDI) Exposure. The acute effects of TDI were first determined by examining 38 workers exposed to levels of TDI below the threshold limit value at the beginning and end of a work day (Monday). Statistically significant decreases occurred in forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak flow rate, and expiratory flow rates at 50 percent and 25 percent of vital capacity. Thirty-four of these same workers were examined on Friday and it was found that the FVC had returned to baseline, the FEV_1 was still depressed, and expiratory flow rates were more depressed. Diurnal variation could not account for these changes. Workers with respiratory symptoms showed greater decreases in FEV_1 than workers without symptoms.

Fluorocarbon Exposure and Cardiac Arrhythmias. An investigation was conducted among certain pathology residents in hospitals who were experiencing repeated episodes of heart palpitations severe enough to require several of them to seek medical care.

The onset of palpitations in the second-year and third-year residents appeared to be associated with the surgical pathology rotation (not assigned to first-year residents). The only procedure performed by the residents peculiar to that rotation was the preparation of frozen tissue sections. Although adequate cryostat machines were available, it was common practice to "speed up" the work of the cryostat machine by spraying the tissue with an aerosol preparation of 100 percent monochlorodifluoromethane (fluorocarbon 22). This preparation had been used in increasing volumes in the pathology department since 1963, mainly because of its convenient form and the fact that it was considered nontoxic. An epidemiologic investigation was undertaken to determine if the apparent association of palpitation with the use of fluorocarbon 22 could be verified. Pathology residents were compared to radiobiology residents.

For the persons having had the onset of palpitation before coming to work in their respective departments, the prevalence and age at onset of the palpitation were similar. The frequency of onset of palpitation after they had been employed remained unchanged for the radiology

department (14 percent) and similarly remained unchanged for the members of the pathology department not exposed to fluorocarbons. On the other hand, half the employees exposed to fluorocarbon 22, 35 percent of those exposed to other fluorocarbons, and 63 percent of those exposed to both preparations had palpitations after employment in the pathology department. Within these exposure categories, this figure represents an increase in palpitation rates of, respectively, 31, 30, and 42 percent. Therefore, persons with any exposure to fluorocarbon preparations at work had a 3.6 relative risk of palpitations as compared to those with no exposure.

There was no association between smoking habits, use of coffee and tea, or the use of medications in relation to the occurrence of palpitation in either of the two departments.

From the data presented, it would appear to be prudent for hospital workers using fluorocarbons in their work, as well as other industrial groups using large quantities of aerosol propellant substances, to reassess their needs and seek suitable substitutes. Certainly, for the pathologist or electron microscopist who works in relatively small, poorly ventilated quarters, alternative procedures or greater attention to ventilation should be explored.

Polymer Fumes. An outbreak of an influenza-like syndrome occurred in a Massachusetts textile mill producing imitation crushed velvet. The production process included the application of fluorocarbon polymer. Seven of 13 employees reported symptoms of cough, fever, chills, aching and weakness, and four reported shortness of breath. Those with the syndrome, identified as polymer fume fever, were all smokers, whereas most of those without symptoms were nonsmokers. Since curing temperatures were too low to produce toxic pyrolysis products, it is believed that exposure was via contamination of cigarettes. The temperature of burning cigarettes, 875° C, was sufficient to produce the pyrolysis products responsible for the symptoms of polymer fume fever. Work practices were changed to require hand washing before smoking, and no illness was subsequently reported. Polymer fume fever should be included in the differential diagnosis of fevers of unknown origin in working persons.

Community Studies on SO_2 and NO_2. Initial surveys of two more communities, the southern tip of St. Louis, Missouri, and Steubenville, Ohio, have been completed. A second survey of the school children with the addition of a new first grade has been completed in Watertown, Massachusetts, and Kingston-Harriman, Tennessee. The final selection has been made for the last two communities, Topeka, Kansas, and Portage, Wisconsin. Preliminary data have been processed from the first three sites.

Central air monitoring stations are in operation in Watertown, Massachusetts; Kingston, Tennessee; and Topeka, Kansas. In St. Louis, data are being obtained from the RAPS station and from a city station.

In Steubenville the NOVAA station is supplying their results. In Portage, data collected at a number of sites in the area by local groups will be used.

In-door-out-door monitoring was done for mass respirable particlates in Watertown last summer and is being repeated this summer. Results from last summer indicated that the values at the central station, in-door and out-door at persons homes, and personnel dosimeters carried by individuals gave similar values. A similar study in the winter showed that the in-door values were different from the others--higher if the family smoked and lower if they did not. Similar summer surveys are being done at Steubenville and St. Louis. It is planned to do these at the various sites and at different seasons of the year to determine how representative the central stations are for the individual's exposures. The usefulness of a NO_2 personal monitor is being assessed.

In November of 1975 there was a period of higher than usual pollution in Steubenville. This presisted for four to five days. Data have been obtained from hospital records (emergency ward admissions and general hospital admissions) for the month of November, 1975 and for the preceeding year. The data are being analyzed to see whether there was any increased use of hospitals during this time and whether it could be related to the levels of pollution.

Bis(chloromethyl)ether. An industry-wide study of the relationship between exposure to bis(chloromethyl)ether (BCME) and lung cancer mortality showed that BCME exposure led to both an increased incidence of lung cancer and to the occurrence of lung cancer early in life (before age 45).

Epidemiological Surveillance Studies

Bladder Cancer in Eastern Massachusetts. A case-control study of bladder cancer among residents of eastern Massachusetts was completed. Interviews were conducted with 470 persons with bladder cancer and with 500 controls selected from the general populations.

As in previous studies, an association was found between bladder cancer and cigarette smoking. The relative risk was 1.89 for males and 2.00 for females.

The relative risk for men ever employed in the rubber industry was 1.63, and was statistically significantly different from 1.00, as was the relative risk of 2.25 for men ever employed in the leather industry. Excess risk was also found among dyeworkers, painters and workers with organic chemicals but these were not statistically significant. It is estimated that about 18 percent of the cases of bladder cancer among men in the study data are attributable to occupational exposures.

Three temporal variables were examined. They were: (1) Age-at-starting, an evaluation of how bladder cancer risk varies with the age at which a man has his first exposure in a hazardous industry; (2) Incubation period, an evaluation of risk as a function of time since first exposure; and (3) duration, risk as a function of time spent in a hazardous industry. These variables are closely interrelated, and statistical analytic procedures were used to assess the independent effects of each.

Prenatal X-ray and Childhood Cancer. In 1962 a study based on 550 children with cancer were reported in a population of 700,000 children born in one of 37 maternity hospitals in the Northeast United States between 1947 and 1954. An association between prenatal exposure to diagnostic X-rays and increased risk of leukemia and other tumors in childhood was observed. A second case-control study was undertaken to see if certain controversial issues could be resolved. These studies have not been as definitive as had been hoped in settling the question of whether there is an association between prenatal X-ray exposure and increased cancer risk.

Leukemia in Patients Exposed to X-ray Therapy for Carcinoma of the Breast and Other Malignant Tumors. This study was designed to confirm a previous study in which patients given intense radiation exposure in treatment of carcinoma of the uterine cervix were found to develop only an expected incidence of leukemia, as determined from general population leukemia incidences. Since radiation leukemogenesis is well established in a number of other studies of a variety of types of radiation exposure, it appears that radiotherapy for cervical cancer involves some different principle of radiation biology. It is hypothesized that high dose therapy to a limited tissue volume in a short time (weeks) results in severe cell damage or cell killing and that this effect outweighs any stimulation to leukemogenesis. If this is correct, radiotherapy for malignancy in general would be expected to be free of a radiation leukemogenesis effect.

A case-control study is being carried out to determine the relative risk of leukemia following radiotherapy for carcinoma of the breast and other malignant tumors. Cases are defined as persons with two independent primary tumors, the second of which is a leukemia or a lymphoma. Controls are selected from patients with tumors of the same primary sites and registered in the same registry as cases.

A total of 1,155 cases are now enrolled from three large cancer registries. These include 128 (11 percent) with primary breast cancers, 235 (20 percent) with primary non-melanoma skin cancers, and 792 (69 percent) with other cancers. Two eligible matched control patients have been found for 94 percent of the cases, and one matched control has been found for an additional 5 percent. Overall results suggest no significant association between the occurrence of second malignancies and prior radiotherapy.

Breast Cancer and Other Malignancies Following Multiple Fluoroscopies. This retrospective cohort study indicates that women who underwent air

collapse therapy for treatment of tuberculosis, and who as a result had considerable X-ray exposure to the breast, had an excess of breast cancer. Among 1,047 exposed women, 41 breast cancers were observed in contrast to 23.3 expected on the basis of Connecticut incidence rates. Among 717 non-exposed women, 15 breast cancers were observed and 14.1 expected. No excess breast cancer among exposed women was seen in women exposed above the age of 28 (8 observed, 8.1 expected).

Neural Tube Defects, Brain Cancer and Possible Association with Lead. To assess the risk of death from CNS tumors following childhood exposure to lead poisoning, a cohort of 383 Massachusetts children treated for lead poisoning at the Children's Hospital Medical Center between 1925 and 1960 was assembled and followed until 1972. Search was made of all Massachusetts death certificates from the date of their discharge. There were no deaths from CNS tumors among the 39 deaths which occurred.

In a separate study, the possibility of association of neural tube defects and maternal plumbism was investigated. Search was made to identify neural tube defects among siblings of this cohort. The study was based entirely on linkage of birth and death certificates. Three hundred ninety-eight siblings were identified. Eighty-one female children of the cohorts were subsequently married. Progeny are being identified and the data analyzed.

Congenital Malformation Surveillance. The possibility of establishing a congenital malformation surveillance and registration system in the Boston area was explored. Abstracts were prepared from birth certificates over a period of one month. It became apparent that such certificates were inadequate as a sole source of malformation data. Plans were prepared for evaluating the various sources of information in maternity facilities--delivery room log books, diagnostic indices, patient records and pathology records. However, during this period interest in this project has declined. The State Department of Health which had expressed considerable interest became unwilling to commit either resources or even moral support to the project. It was decided to table further work on this project--at least temporarily--in favor of more limited studies focused on the environmental determinants of specific categories of malformation. Discussions are underway with the Boston Hospital for Women, to interview women at their first prenatal visit. Among the information to be obtained will be occupation of the parents. If this surveillance system develops into an ongoing project, the large volume of data needed to assess the association between parental occupation and birth defects will become available.

Proportional Mortality Among Meat Packers. An excess number of deaths from cancer of the prostate and of the bladder was seen among a group of 900 deceased meat packers. After review of the work histories of these persons, no common exposure was found. In an attempt to assess this finding, county-specific mortality rates are being assembled in counties where meat packing is a major operation. The possibility of repeating the study in another population of meat-packers is being considered.

Death in Boston. A pilot study is underway to determine the feasibility of studying environmental factors using town listings and death certificates in Massachusetts. Three basic pieces of information are being collected: occupation, place of residence and ethnic group. Two approaches are being tested:

1. Town list approach: 8,000 names have been abstracted from Boston Town lists between 1925 and 1965. These persons are being followed using death certificates, town lists, and Polk directories. A cohort analysis will be used to compare observed and expected deaths.

2. Death certificate approach: About 7,000 death certificates from Boston for 1970 are being abstracted. A proportional mortality analysis will be used to compare observed and expected deaths.

Analysis of Relative Survival and Proportional Mortality. A computer program utilizing cause-age-time-sex-race specific mortality or incidence rates was developed. The program is used to calculate the expected number of deaths or cases of disease in a cohort. Also, among a group of persons known to be dead, the number expected to have died of each cause can be calculated using proportional mortalities. Mortality rates based on United States vital statistics were assembled for 57 categories of cause of death for white males, white females and black males for each five-year age and time period from 1925 through 1974. Incidence rates from the Connecticut Cancer Registry were assembled. The computer program is general and can be modified to use other sets of rates in determining the expected number of deaths in any study population.

This program is undergoing continual revision. A feature enabling direct standardization of rates has been added. A modification to use data coded to the Eighth Revision of the International Classification of Diseases is in progress.

Cohorts of workers and, in some cases, the general public have been established and are being kept under surveillance in connection with exposure to the following gases and vapors:

1. Polycyclic Hydrocarbons. Analyses are being continued of data relating to the mortality experience of roofing workers in the United States between 1960 and 1971. Significant cancer risk has already been established for this group. The study will continue through 1976 and various roofing materials now in use will be analyzed for polycyclic hydrocarbon content.

2. Organohalides.

   a. PCBs. A cohort consisting of 325 employees of the Capacitor-Transformer plants of General Electric at Fort Edward and Hudson Falls, New York, has been

established. The mortality experience of these workers is expected to provide the first information regarding health effects of long-term PCB exposures in humans.

b. PBBs. A large scale survey has been made of 1,000 Michigan farmers and their families exposed to varying amounts of PBBs resulting from an inadvertent contamination of livestock feed. Results from the studies so far indicate several types of pathology, including possible neurological damage in some individuals.

c. Vinyl chloride. Three cohorts of approximately 1,200 vinyl chloride polymerization workers are under observation. A prospective analysis of histological and pathological effects of exposure will be made. Long-term clinical studies of the effects of vinyl chloride have been undertaken to stratify appropriate samples for re-examination and evaluation of serial changes and the possible progression of disease in the absence of further exposure.

d. Trichloroethylene. Trichloroethylene at very high doses by gavage has been demonstrated to be carcinogenic in laboratory animals. A cohort of retired dry-cleaning workers who were exposed at one period has been identified and the mortality experience is being recorded.

e. Anesthetics. A number of studies have been interpreted as showing increased risk of cancer among anesthesiologists. In cooperation with the American Association of Nurse Anesthetists and the American Society of Anethesiologists, cohorts are being established for prospective tracing of cancer incidence from the 1950s through 1975.

f. Styrene. Analysis of data is in progress on the mortality and cancer experience of a large cohort of styrene workers through 1975. Results of the study are expected soon.

g. Solvents. A number of neurological and behavioral abnormalities have been found among workers in the painting trades exposed to a variety of solvents. Field studies of solvent-exposed workers are being conducted with particular attention to behavioral effects.

Records of some 6,000 of these painters are being traced and death rate and incidence of cancer will be compared among workers engaged in trades such as general painting, sandblasting, glazing, drywall taping, paint making, bridge painting and other activities in these trades. These studies could also have implications in the general population for individuals who do their own painting, etc. around the home.

h. Nitrosamines. Extensive experience has accumulated regarding carcinogenicity of nitrosamines in animals. To obtain commensurate information in a human population, a group of approximately 350 individuals has been identified who worked with dimethylnitrosamine in the rocket fuel facility of the FMC Corporation in Baltimore. Most of the workers were exposed subsequent to 1960; thus it is not expected that definitive data will be available on carcinogenic experience, mortality, etc. for several years. However, the group is being kept under surveillance prospectively to assess the carcinogenic potential of this nitrosamine in humans.

Development of Air Sampling and Analytical Methods

Nitrogen Oxides. Early experience in aerometric studies indicate that improved air sampling and analytical methods were required for personal sampling of nitrogen dioxide. A satisfactory solution to this problem has been found, permitting development of dose information on exposed personnel in a variety of industrial and air pollution studies. The method involves collection of the sample on porous polymer beads, gas chromatography (detection limit = ppb) with a flame ionization detector, and detection of nitrites and/or nitrates with ion-specific electrodes or by polarography.

Carbon Monoxide. To provide a better estimate of exposure of the workers such as traffic officers who are quite mobile and are, therefore, subject to contaminant concentrations which may vary both temporally and spatially, a personal sampler has been developed which provides a precise estimate of the average CO concentration in a four or eight hour integrated sample.

The sampler consists of stripping beds for removal of water vapor and CO_2 from the sample air, a CO sampling tube which oxidizes CO and collects the resultant CO_2, and a personal sampling pump operating at 0.5 lpm for four to eight hours.

A multi-contaminant sampler was designed for use during structural fire-fighting to determine exposure to the spectrum of gases which may occur from pyrolysis and/or combustion of various materials. Six gases, O_2, CO_2,

CO, NO_2, HCl, and HCN can be determined. Carbon dioxide has been reported in high concentrations in experimental burns. Nitrogen dioxide might be expected at fires through fixation of atmospheric nitrogen and, to a lesser extent, from the oxidation of nitrogenous materials. Hydrochloric acid would arise from the pyrolysis and combustion of PVC-containing plastics frequently encountered in structural fires. Sources of hydrogen cyanide are wool and plastics containing urethanes, acrylonitriles or polyamides. The system was designed to permit incorporation of other contaminants at a later time.

Mechanistic and Etiologic Studies of Gases and Vapors

Ozone and NO_3. It is now clear that exposure for as little as two hours per day to high smog concentrations of NO_2 and O_3 together causes changes in the epithelium of rat lungs (small airways and adjacent alveoli, in particular) after several months. Concentrations of NO_2 in smog varies from about 0.8 to 1.0 ppm, whereas O_3 varies from about 0.5 to 0.9. Stimulation of erythrogenesis is manifested in rats as polycythemia after about six months of exposure for two hours daily. Currently, lower concentrations applied for two hours daily are being followed.

The epithelial changes following exposure for two to four hours daily to the mixture of 0.9 ppm each of NO_2 and O_3 were confirmed by electron microscopy, which revealed the ciliated nature of cuboidal cells lining proximal walls of alveoli adjacent to respiratory alveoli. Lysosomes were altered also.

The functional determination of small airway disease in its early stages, as induced in the rat by exposure to oxidants, was found to depend considerably on the degree of initial distension exhibited by the excised lung and the relative amounts of secretion and debris in the lumens of such airways as well as on the more enduring structural "stiffening" of terminal and respiratory bronchioles and ducts. Thus, the significance of frequency dependence of compliance and resistance may be too complex for ready evaluation.

The biochemical mechanism of action and the responses of pulmonary tissue to the oxidants NO_2 and O_3, respectively, differ in several ways by relatively arbitrary criteria such as survival and degree of injury to lungs. However, estimates of toxicity indicate that O_3 is about 10 to 20 times as injurious as NO_2. This difference is important because excesses of O_3 above natural background levels derive largely from atmospheric NO_x, and both O_3 and NO_2 appear in similar concentrations in photochemical smog.

By labeling cells with tritiated thymidine before they divided, it was deduced that Type 1 alveolar epithelial cells newly replacing those previously injured by O_3 are relatively resistant to that gas upon reexposure, whereas older cells (nonlabeled) are vulnerable. Also, the initial tolerance developed to a particular concentration can be overcome

by raising the concentration. These effects are quantified by counting labeled progenitor Type 2, intermediate, and daughter Type 1 cells as a function of time.

In selected samples of lung, the thickness of the air-blood barrier was measured electron microscopically when its three components could be identified. It varied up to 15 to 20 times the average control value of about 0.45 μ, depending on the animal's prior history of exposure, the site selected, and the preparation of the lung. Dense intracytoplasmic granules of unknown function were observed to exist only in pulmonary bronchiolar arteries.

As demonstrated qualitatively by histochemistry, glucose-6-phosphate dehydrogenase (G-6-PDH) in fresh, frozen lung resides mainly in terminal bronchiolar and Type 2 alveolar epithelial cells and is exaggerated somewhat in proliferating cells. Macrophages also stain for the enzyme.

Exposure of rats and monkeys to 0.5 ppm ozone, eight hours a day for seven days, caused no changes in glutathione or glutathione peroxidase in circulating erythrocytes. Exposure of rats and monkeys to 0.8 ppm ozone, eight hours a day for seven days, caused changes in LDH iosoenzymes in lungs and plasma of rats, but total LDH changed (increased) only in lungs. No significant changes were detected in lungs or plasma of monkeys.

Early attempts to characterize the selenium-containing prosthetic group of glutathione peroxidase using X-ray photoelectron spectroscopy, sulfhydryl and organomercurial gels, and chromatography were inconclusive but compatible with a seleno-trisulfide model of a prosthetic group.

The potentiating effect of vitamin E deficiency on ozone-induced pulmonary damage in rats was further evidenced by the finding that mitochondrial enzyme activity (succinate oxidation) increased over control by 38 percent and 25 percent by seven days of exposure to 0.2 or 0.1 ppm ozone, respectively, in marginally vitamin E deficient rats (11 mg/Kg dietary supplement) as contrasted with 17 percent and no change, respectively, by the same exposures in rats given 66 mg/Kg vitamin E in the diet. Determination of vitamin E by thin-layer chromatography resulted in poor reproducibility (except for adipose tissue); subsequently, a successful, but laborious, analysis was achieved with a radioisotopic dilution method using gas chromatography. Currently, deuterated vitamin E is being substituted as the isotopic diluent for assay by gas chromatography-mass spectrometry.

Vitamin A has been assayed biochemically on extracts of lungs, blood, alveolar macrophages, and livers of whole oxidant-exposed and unexposed rats. Animals varying in age from 7 to 77 weeks and exposed for either 3 or 323 days to 15 ppm of NO_2 or for 3 days to 0.9 ppm of O_3 revealed lung vitamin A content that usually was equivalent to that in controls. Because of variable degrees of interstitial edema and the variety of cell types in exposed tissue, vitamin A content is being recalculated on the basis of units of DNA. Nevertheless, the total content in 2- and 20-month-old rats exposed for 3 and 322 days, respectively, tended to be reduced.

Immunochemical analyses of normal lung lavage fluid from mice revealed 10 or more serum proteins, including albumin, IgG_1, IgA, transferrin, and α and β globulins. IgM was not detected. Tentative conclusions are that IgA does not quantitatively dominate the antibody populations in respiratory secretions of normal mice, in contrast to some species, and little IgM synthesis occurs locally in normal mouse lungs.

Interferon production by *in vitro* cultures of tracheal epithelium and alveolar macrophages from mice continually exposed to 0.8 ppm ozone was assayed. Up through 10 days of exposure there was no lessening of interferon induction in cultured explants of tracheal epithelium by Newcastle disease virus or poly I:C. Inhibition was detected, however, in epithelium taken from mice exposed for 11 days or more. In contrast, there was no influence of ozone exposure on the capacity of alveolar macrophages to produce interferon *in vitro*.

The role of lysosomal enzymes in the inactivation of inhaled *S. aureus* by alveolar macrophages was studied in rats exposed to 2.5 ppm ozone for five hours. There was absence of histochemical evidence for acid phosphatase and beta glucuronidase in macrophages of ozone-exposed lungs, particularly in those containing bacterial microcolonies.

The lungs of nursing mothers appear to respond to oxidants in an exaggerated fashion, whereas lungs of their offspring below 10 days of age, as well as those of prenatal animals, show little or no tissue response to continuous exposure to either 15 ppm of NO_2 or 0.9 ppm of O_3. However, nursing rats older than 10 days do respond to NO_2 with loss of cilia in the terminal bronchioles and with an elevated rate of division of bronchiolar epithelium, but not to O_3, which induces such changes only after 21 days of age. At that time (weaning), Type 1 cells in proximal alveoli become affected by both gases and are renewed by division of Type 2 cells. The pathogenesis, including the appearance of macrophages, is augmented with time until about 40 days of age.

G-6-PDH becomes apparent histochemically in the apical portions of epithelial cells lining the developing airways on the fourteenth day of gestation in the rat. The enzyme is detectable in Type 2 cells two to four days later and becomes maximally evident postnatally at 40 days of age. These observations correlate with biochemical measurements of pulmonary tissue.

Although equating doses between NO_2 and O_3 is rough and rather arbitrary according to the criteria selected, changes in G-6-PDH activity following three days of exposure to each gas were compared. After 15 days of age, the response to NO_2 entails a doubling of enzyme activity over control levels, and, after 20 days of age, the response to O_3 is half of that induced by NO_2. Also, the effect of the former is less persistent.

The relationship between competence of lungs to respond to the oxidants and their states of neonatal maturity proved to be unaffected by either high inositol content in the diet of the newborn or the caloric properties

of a high-fat diet simulating mother's milk. Induced elevations of enzyme activity in pulmonary tissue are not attributable to blood.

Combinations of Pollutants. A study is being conducted on the effects of combinations of air pollutants on respiratory and cardiovascular disease in laboratory animals under carefully controlled conditions. By means of a programmed monitoring system with feed-back control, levels of the pollutants are maintained at preselected levels. At present, the mixture consists of SO_2, NO, NO_2, CO, CH_4 and $(NH_4)_2SO_4$ aerosol. The levels are considered typical of urban air pollution, high or episodic exposure, and exaggerated or emergency exposure.

In normal rodents (rats and hamsters) which were placed in the exposure rooms about two years ago, there was no correlation between the level of air pollution and mortality rates. Most of the animals have now died (expected life span). Growth of the animals was essentially normal. Hematologic parameters (hematocrit, hemoglobin, carboxyhemoglobin) measured at various intervals during the two years indicated compensatory increase in red blood cell production as a result of heavy air pollution. The effects of air pollution on atherosclerosis were studied in rats fed a thrombogenic diet. Mortality was significantly higher in the heavily polluted air, but atherosclerotic lesions were not as severe as expected. A high incidence of urolithiasis (85 percent) was observed in rats fed the thrombogenic diet, which may be related to the high calcium content of their drinking water (source from limestone wells). Another experiment is now under way to test this possibility.

Renovascular hypertension was induced by constriction of the left renal artery in 48 male rats. Sham operations were performed on another 48 rats for control purposes. Premature death in the hypertensive rats has clearly resulted from air pollution and mortality is directly related to the level of air pollution. Additionally, length of survival of hypertensive rats showed a highly significant correlation with severity of hypertension and was inversely related to the level of air pollution.

Hamsters injected intratracheally with elastase to produce emphysema showed no differences in severity of emphysema or in mortality, nor were there any differences in death rate between the emphysematous and control hamsters exposed the combination of pollutants tested.

Carbon Monoxide (CO). A series of experiments were carried out to determine the effects of low level (150 ppm) prenatal CO exposures in rats. The results indicate that offspring which were exposed to CO in utero had a lower growth rate and activity level than controls through 21 days of age. Endogenous brain norepinephrine and dopamine levels of the experimental animals were normal, but at one and four days of age following injection of L-DOPA, they exhibited lower brain dopamine levels than controls with the same treatment. Experimental animals also had lower brain protein content than controls. These findings indicate that chronic prenatal exposure to CO, at concentrations that approximate carboxyhemoglobin twice that of the average human cigarette smoker, affects central nervous system development.

When neural and humoral effects have been removed, the myocardial response to CO is similar to the low oxygen response at equal arterial oxygen content; that is, the increases in blood flow, reduction in myocardial force of contraction, and total vascular resistance are similar. While arterial resistance decreases less with carbon monoxide, venous resistance decreases more. Oxygen consumption falls more with carbon monoxide than with low O_2 exposure suggesting that CO causes in addition to its binding with hemoglobin some reduction of metabolic activity probably through cytochrome oxidase inactivation.

It was found that cerebral circulation compensates for both hypoxic hopoxia and CO hypoxia by increasing its blood flow equally for a given reduction in arterial oxygen content. This cerebral vasodilation is not altered following chemoreceptor denervation. It appears that the brain increases its blood flow in response to its oxygen need with both types of hypoxia in order to maintain constant oxygen consumption. The chemoreceptor is not responsible or necessary for this cerebral vasodilator response. Low levels of CO (below 10 percent COHb) still elicit the vasodilator response and there does not appear to be any threshold level below which this compensatory vasodilation occurs. Studies on the cerebral response to the administration of cyanide hypoxia show that the brain vessels dilate to increase their blood flow in this treatment also. This response is also independent of the carotid chemoreceptors.

In the past year it was observed that both CO and common inhalation anesthetics interfere with placental oxygen delivery to the fetus to a significant degree. At a blood carboxyhemoglobin level of 8 to 10 percent (equivalent to that of a heavy cigarette smoker) placental oxygen transfer was reduced by 50 percent. Inspiration of 1 to 2 percent methoxyflurane (an anesthetic) also reduced placental oxygen transfer by 30 percent. These findings may help to explain the well-known adverse human health effects of smoking or exposure to inhalation anesthetics during pregnancy.

Benzene. Continuing studies on benzene indicate that the compound depresses lymphocyte levels of the test animals. Granulocyte levels, however, were unaffected. Anemia, although not statistically evident in the rat, was present in both strains of test mice at both exposure levels. Lactic acid dehydrogenase levels were, at times, elevated in the rats exposed to 300 ppm benzene. Glutathione levels were not found to be different in the rat studies but showed a depression in mice which paralleled the drop in red cells. Chromosome studies were inconclusive except that the A.K.R. mice exposed to 300 ppm consistently showed a higher percentage of chromosome breaks and hypodiploidy. Red cells from test rats and mice showed slower hemolysis rates when placed in glycerol solutions.

Histological evaluations of bone marrow sections showed that test rats and mice at 300 ppm have hypoplasia. The hypoplastic condition is much more severe in mice and also appears earlier upon exposure to benzene.

Gas chromatographic studies of tissues from mice exposed to 100 and 300 ppm benzene show that unmetabolized benzene reaches the bone marrow. Studies are in progress to determine clearance rates. Preliminary results indicate faster rates for mice than rats, suggesting more rapid metabolic activity in this species. These studies demonstrate that benzene induces aplastic anemia in test animals, and depression of the immune response mechanism. There is no evidence, as yet, of benzene-induced leukemia in any of the studies.

Studies have also demonstrated benzene inhibition of rabbit reticulocyte heme synthesis and delta-aminolevulinic acid synthetase.

Organohalides. Studies recently demonstrated that energy producing devices utilized in the operating room, e.g., electric cauteries and X-ray machines, result in the oxidation of the anesthetic gas nitrous oxide to nitric oxide and nitrogen dioxide. Similar reactions involving halogenated hydrocarbon anesthetics may produce compounds responsible for the increased incidence of spontaneous abortion in female operating room staff as well as other matagenic and teratogenic effects possibly associated with employment in this area.

Studies of the multiple environmental factors involved in the induction of lung cancer involve specific carcinogens and a variety of agents which may modify the manner in which the lung responds to inhaled materials. In previous studies, the role of the respiratory irritant in the multifactorial nature of lung carcinogenesis has been demonstrated in rats exposed to a combined atmosphere of nitrogen dioxide (NO_2) and benzo(a)pyrene (BP). An interesting finding was an angiosarcoma of the liver.

It was found that lung carcinoma incidence produced by intratracheal intubation of BP in hamsters was increased by concurrent exposure to SO_2. Sulfur dioxide did not increase the lung cancer incidence of methylcholanthrene, but a sharp reduction of tumor induction time was observed (800 to 400 days).

Initial studies have demonstrated that bis(chloromethyl)ether is a carcinogen in mouse skin. Follow-up studies showed it to be carcinogenic in rodent lung as well. In a similar manner, mouse skin carcinogens epichlorhydrin and dimethylcarbamoyl chloride are currently being examined by inhalation exposure in rats and recent data show both to be carcinogenic. Initial range-finding studies have been completed with dimethylcarbamoyl chloride; these have included LC_{50} and limited exposures ranging from 100 to 1 ppm. A chronic study with rats exposed to 1 ppm has resulted in a high (almost 100 percent) incidence of squamous cell carcinomas of the nasal cavity. An additional study at a level of 0.3 ppm is now is progress.

Recent evidence suggests that chlorinated ethers may be formed spontaneously in the atmosphere from the combination of hydrochloric acid (HCl) and formaldehyde (CH_2O). Current studies appear to verify this reaction. Chemical studies in saran bags and inhalation chambers using high concentrations of HCl and CH_2O have demonstrated ppb levels of bis(chloromethyl)-

ether and low ppm levels of active alkylating components. The primary ingredients in the reactions have widespread industrial and laboratory use, representing a potential hazard to a very large part of the population.

Suspended Particulates (Aerosols)

Epidemiological Studies

Over the past five years, several populations have been studied for evidence of occupational lung disease. The ultimate goal is to develop dose-response data for the exposures and health effects.

A cross sectional approach is to be used. This simply determines the health status of a group of employed workers, comparing it with the health status of a control population. Past exposure is estimated by grading job exposures and multiplying job exposures by years in that job. In some cases, a lifetime dose can be reasonably estimated.

Granite Workers. Seven hundred ninety-two granite shed workers were examined by this technique. For dust exposure, restrictive disease was evident, while for smoking, obstructive disease was produced. The relative contribution of each is quantifiable. In addition, a dose-response relationship was observed between lifetime dose and changes in both pulmonary function and X-ray. Increase in dust exposure correlated with increase in size of rounded opacities and profusion. Irregular opacities were related more to smoking than to dust. Forced vital capacity (FVC) was lower for people with abnormal roentgenograms and decreased with greater profusion. Residual volume (RV) increased with smoking but not with dust exposure. No trend was shown for total lung capacity (TLC).

Asbestos Workers. A survey was initiated to assess disease in workers in a wall board manufacturing operation. Fifty-seven workers were examined by questionnaire, pulmonary function tests, and limited physical examination. Definite evidence of excess pulmonary disease was found. The prevalent respiratory syndrome was compatible with asbestosis, was highly correlated with length of asbestos exposure, and could not be explained by smoking or other known environmental factors.

Cohorts of other workers exposed to asbestos are also under surveillance. These include several groups:

1. Indoor pollution. From 1959-1973 most high-rise buildings were constructed with sprayed asbestos fireproofing. It has been found that there is significant opportunity for indoor asbestos air pollution in these buildings. Measurements are being made of the air levels of contamination as a guide to projected control measures, particularly in schools.

2. Neighborhood pollution. Though the risk of mesothoelioma and bronchogenic carcinoma in workers exposed to asbestos has been established, the degree of risk to non-workers in the vicinity of asbestos sources has not been determined. Thus a cohort has been established of all residents in the neighborhood of an asbestos plant in Patterson, New Jersey, which operated in 1942 and 1943. The individuals have been traced through 1975 along with a control cohort in the same city away from the vicinity of the plant. Analyses of the mortality and cancer experience of the two groups are underway.

3. Insulation workers. Studies of mortality experience of asbestos insulation worker in the New York area in relation to smoking habits show that cigarette-smoking asbestos workers had approximately eight times greater risk than non-smoking workers and 92 times the risk of men of the same age who neither worked with asbestos nor smoked cigarettes.

   It is known that cessation of smoking decreases the future risk of cigarette smokers to cancer. A study has been launched to determine if cessation of smoking is beneficial in reducing the cancer incidence in asbestos workers.

4. Mine and mill workers. In 1974, a cohort of 564 long-term Quebec asbestos mine and mill employees was identified and their mortality experience was calculated and compared with that expected in the general population of Canada. The overall mortality experience was not very much different from that of asbestos insulation workers and of asbestos workers in the United States in a factory which largely used fibers from this area. The data are expected to permit comparison of the mortality experience of workers exposed to chrysotile asbestos under varying circumstances of mining, milling, product manufacturing, and product use.

   Studies are underway to assess the possible role of asbestos in cancer of the rectum, sigmoid colon, descending colon, transverse colon, ascending colon and cecum in insulation workers.

   In a prospective study of the mortality experience of insulation workers in the United States and Canada, it has been found that by the end of 1974, 1,600 deaths had occurred, 20 percent due to bronchogenic carcinoma and more than 5 percent to pleural and peritoneal mesothelioma. Other causes of death were increased, including cancer of the esophagus, stomach, colon, and rectum.

5. Other asbestos workers. Studies are being conducted to develop information as to the optimum design of a clinical surveillance program of asbestos insulation workers, including sputum cytological studies and clinical X-ray examination. Similar clinical surveillance is being maintained on cohorts of amosite factory workers, shipyard workers, maintenance workers, brake repairmen, chrysotile asbestos miners and millers, and other groups with known exposure to asbestos.

   Although the major concern regarding taconite mining operations of the Reserve Mining Company has been contamination of the waters of Lake Superior, a more localized problem might be the contamination of air with amosite fibers from the crushing and pelletizing operations of the company. These operations have been found to result in concentrations of a million fibers/meter^3 or air in the vicinity. Long-term average air concentrations range from 100,000 to 500,000 fibers/meter^3. Evaluation of possible health effects of the fibers at these levels is underway. The outcome of the study should have a bearing on control measures.

   Initial studies have provided evidence of asbestos disease, including mesothelioma, among brake repair workers. There are over one million such workers in the United States.

   An instance of mesothelioma has been reported in a toll-booth operator in Connecticut. Investigations are underway on the feasibility of establishing a cohort of toll-booth operators to provide additional information on the broad question of asbestos exposed and risk in various occupations and settings.

6. Wives and children of asbestos workers. Studies are being conducted of pulmonary changes of the wives and children of asbestos workers. Extensive pulmonary parenchymal fibrosis does not occur among household contacts of asbestos workers. However, at the low levels brought in on clothing, etc., some parenchymal fibrotic changes do occur and pleural changes (fibrosis, calcification) are also seen. These studies are being conducted on families of workers employed in the plants during 1941-1954, i.e., 22 to 32 years after exposure.

   Among the nearly 700 family contacts of asbestos workers first X-rayed, approximately one-third have one or more abnormalities. When the survey is complete, results will be analyzed in terms of

duration of employment of the worker, duration of household exposure, smoking habits, sex, age, laundering habits, etc.

7. Hardmetal workers. Increased lung cancer among hardmetal miners has been attributed to a number of factors, particularly silicates. There is a question as to whether silicates exhibit the biological properties ascribed to asbestos. In preparation for biological studies, the silicates from these mines are being characterized for correlation with future biological studies.

Physical Properties of Minerals. The surface properties of biologically active minerals are being studied in relation to their pathological potentials. On the basis of preliminary studies with chrysotile and amosite asbestos, different surface properties appear to produce quite different membrane effects. Electron and proton migration at the surface of minerals have been observed to influence free-radical stability in in vitro test systems.

Tissue Analyses for Minerals. Electron microscopic studies are being conducted on experimental and human mesothelioma to improve diagnostic procedures and accuracy. Ongoing work permits concomitant qualitative and quantitative minerological analyses of lung tissues in many cases and correlations of occupational, medical and smoking histories. In many instances, the diagnosis is not clear at the time of biopsy. An initial diagnosis of mesothelioma sometimes turns out to be inaccurate. Thus, better procedures are needed for distinguishing mesothelioma from bronchogenic carcinoma and other tumors by electrom microscopy. Analyses are underway of lung tissues of workmen in the following industries: abrasive soap manufacture, talc industry, coke oven workers, asbestos workers, zeolite exposure in the petroleum industry, tunnel and caisson workers (silicosis), sandblasters (accelerated silicosis), vermiculite and silica exposure and fibrous glass exposure. Data on particle shape, size distribution, and chemistry are being accumulated. All data will eventually be evaluated and correlated with specific diseases.

In general, spirometry has been the most widely used pulmonary function test to detect lung impairment in environmental surveys. Studies have been conducted to determine if the carbon monoxide diffusion test might be practical in clinical field surveys and provide information on earlier damage in the lungs than that revealed by spirometry. Using both automated and conventional single breath CO diffusing techniques, initial studies have not demonstrated the CO tests to be any more sensitive than the usual vital capacity estimation, even though it does appear feasible for field studies.

Analytical Methodology for Particulate Quartz. Quartz can be determined by chemical methods, differential thermal analysis, microscopic examination, X-ray diffraction, and infrared spectrophotometry. The only

reliable chemical method with adequate sensitivity involves the separation of quartz by phosphoric acid treatment and eventual determination colorimetrically as molybdosilicate or molybdenum blue.

X-ray diffraction is among the most promising methods, but it has some drawbacks. The equipment is expensive and offers potential radiation exposures which should be constantly monitored. Linearity of response is restricted to a limited mass range of dust in a sample, and interference may be expected from some constituents of many types of samples. Efforts have been centered on the infrared spectrophotometric procedure which has been applied for occupational health studies in both the granite and talc industries.

1,1'-Azobisformamide (Kempore). This foaming agent is an offending factor in a series of complaints from workers grinding and sizing the powder. In order to assess worker exposure it was necessary to develop a method to determine the compound collected on membrane filters. Since the material dissolves readily in dimethylsulfoxide (4.3g/100g DMSO at 20° C) a spectrophotometric procedure has been developed which is quite satisfactory.

Portable Sampler for Air Pollution Studies. In a series of studies, it was necessary to conduct air sampling to obtain data on the exposure of traffic officers, toll booth, and tunnel operators to pollutants from automobiles. The contaminants chosen to reflect exposure were nitrogen dioxide, carbon monoxide, total hydrocarbons, mass respirable particulates, and particulate lead. Since sulfur dioxide is a pervasive contaminant in the city, it was also included. A suitable personal monitoring procedure permitted the air sampling program to be initiated concurrently with epidemiologic studies and it provided valuable field data.

Mechanistic and Etiologic Studies of Suspended Particulates

Sulfur-containing Particulates and Mists

In studies on chronic inhalation exposures of hamsters to sulfuric acid mists at 100mg/M^3, a number of respiratory carcinomas have been observed in the multiple exposure groups. These include squamous cell carcinomas, adenocarcinomas and mixed carcinomas.

In order to evaluate the problem resulting from formation of intermediate sulfur oxides and/or interaction of these products with particulate materials, a catalytic converter has been developed. Currently, various catalysts and their efficiencies of conversion are under study. Future studies will include the effects of humidity and the introduction of particulate materials such as metal oxides and carbon. Animal studies will then be designed to characterize the toxicity of the suspected product mixtures.

Studies are being continued to characterize the physiological effects of short-term inhalation exposure to submicron aerosols of H_2SO_4 and $(NH_4)_2SO_4$

on healthy adult donkeys. Particulate sulfates are a class of pollutant which may produce significant health effects, and sulfuric acid and ammonium sulfate are often major mass components of the submicron mode of the ambient aerosol in both urban and rural areas. The response is characterized in terms of changes in pulmonary flow resistance (R_L), pulmonary compliance (C_L), regional deposition and bronchial clearance pattern of γ-tagged, monodisperse, insoluble ferric oxide aerosols.

One-hour exposures to $(NH_4)_2SO_4$ at mass concentrations up to 4.3 mg/m^3, and to H_2SO_4 at concentrations up to 3.0 mg/m^3 did not produce any statistically significant effects upon R_L or C_L. On the other hand, one-hour exposures to H_2SO_4 at concentrations of 0.42-0.64 mg/m^3 did produce significant short-term slowing of bronchial clearance in all four donkeys. At lower concentrations, the animals showed individual variability in response, i.e., all did not respond at comparable exposure levels, and there appeared to be a wide variability in the intensity of response among the individual animals. The effect upon clearance did not become apparent until 30 minutes after initiation of acid exposure. Two of the donkeys exhibited a progressive reduction in their control clearance rate during the course of the series of one-hour exposures to the submicron H_2SO_4 aerosols.

A series of regional particle deposition studies have been completed on 44 healthy human volunteers. *In vivo* measurements of total, alveolar and tracheobronchial deposition were obtained for particles of 0.2 and 5 μm. For electrically charged particles, there was an enhancement of deposition due to charge in both *in vivo* and in the hollow cast experiments. Analysis of the particle deposition data included an evaluation of the validity of various predictive deposition equations. The equation of Cheng and Wang of Syracuse University was found to be the best in predicting impaction deposition in the airways and the widely used equation of Gormley and Kennedy was best for diffusional deposition.

Behavioral methods were used in determining the effects of catalytically converted automobile emissions, H_2SO_4 mist and CO. Converted exhaust contains more acid than untreated control exhaust. Exposure to pure H_2SO_4 mist or CO caused relatively minor behavioral changes only. Rats exposed to untreated exhaust were observed to have depressed body weight, food and water intake, and decreased spontaneous motor activity.

Physiological Studies of Pulmonary Defense Systems

Many projects supported by NIEHS research grants depend on the reliable and reproducible production of a submicronic insoluble aerosols. Although the Wright Dust Reeder and similar generators to resuspend preexisting dusts is still used, experience suggests that generators are able to make particles *de novo* more dependably.

Iron oxide was selected as a convenient test aerosol since it can easily be quantified by standard techniques of chemical analysis, such as atomic absorption, colorimetry, or spectrophotometry visible in unstained tissues

as a reddish brown pigment. It can also be visualized histochemically by means of the Prussian blue reaction for iron. In addition, certain types of workers, such as arc welders, silver polishers, iron grinders, and the miners and millers of hematite and magnetite ores have significant exposures to iron oxide dust of fumes.

The aerosol size distribution was estimated using electron microscopy and an Andersen Dust Sampler. The particles appeared as irregular, linear agglomerates of many smaller particles (0.01 to 0.03 μm diameter). The size distribution was approximately log normal with a median diameter of 0.35 μm and a geometric standard deviation of 2.03.

Lysosomal enzyme activity in pulmonary macrophages lavaged from iron-exposed and control rabbits have been studied. Macrophages were harvested from groups of animals by multiple lung lavage immediately following the exposure to ferric oxide aerosol and at 12, 18, and 24 hours thereafter. Levels of β-glycerophosphatase showed little variation, never falling more than 25 percent below controls. Cathepsin D specific activities were unchanged at 0 and 12 hours, but were at least 50 percent lower than controls ($P<0.005$) at 18 and 24 hours. Although both enzymes are lysosomal, their patterns of response are not coincident. Enzyme levels may reflect loss and degradation of enzyme as well as synthesis; influx of immature macrophages with lower enzyme levels would tent to minimize adaptive increases stimulated by endocytosis. The specific activities of other acid hydrolases, i.e., p-nitrophenyl phosphatase, β-glucuronidase, β-galactosidase, and N-acetyl-β-glucosaminidase, do increase by 18 and 24 hours after iron oxide exposure. The particles appear to selectively stimulate some lysosomal enzymes, but not all.

It was anticipated that there might be an alteration in the distribution of the lysosomal enzyme activities within the cells which have ingested the Fe_2O_3. To examine this possibility, a series of experiments utilizing sucrose density gradients to fractionate cell homogenates was performed. There was found to be an increase in density of lysosomal enzymes from 1.21 in controls, to 1.25 in Fe_2O_3 containing cells. During the 24 hours following aerosol exposure there is a progressive increase in enzyme activity in the more dense, iron oxide containing region of the sucrose gradient, and a decrease in activity in the lighter, primary lysosome region, indicating fusion of primary lysosomes with iron oxide filled phagosomes to form secondary lysosomes. This information on the alevolar macrophage response to iron oxide, a relatively non-toxic particle, provides a background from which to assess the responses of these cells to more toxic inhaled particulates.

Particle Clearance. The history of particle clearance was studied in lungs of mice serially sacrificed at intervals up to 14 months following single exposures to an aerosol of submicronic, particulate, iron oxide used as a similitude for atmospheric dust. The iron particles settle extensively but not uniformly on pulmonary alveolar surfaces. Clearance is centripetal and involves two mechanisms, an extracellular mechanism fed by fluid currents sweeping across the surface, and a cellular mechanism principally involving alveolar macrophages. In the early post exposure

period both actively remove deposited particles predominantly through the pulmonary airways. By 24 hours uncleared residues become ingested and clearance thereafter results mainly from cellular action. A chronic phase of clearance begins when deposited particles become sequestered in macrophages of pulmonary connective tissues. These cells are reached by several routes, not least by crossing the bronchial epithelium. Particle clearance from these macrophages is very slow, and residue-containing cells eventually congregate in lymphoid tissues surrounding major bronchi.

This study shows that the greater part of uncleared material in the lungs finds its way by lymphatic and other routes into pulmonary connective tissue macrophages located primarily in the investments of the larger airways. Apparently, in retaining undigestible but biologically active (radioactive, carcinogenic) particles, the intracellular digestive (lysosomal) system of the connective tissue macrophages remains chronically activated, and in concentrating this material these cells foster a "catabolic" environment locally, which helps to explain why some types of emphysema are centriolobular in origin, and why in many species the main pulmonary carcinoma is bronchogenic.

It was observed that infrequently, and usually some weeks after mice had been given a single three-hour exposure to an iron oxide aerosol, finely divided iron oxide and/or its metabolic sequellae, ferritin and hemosiderin was observed in the cytoplasm of ciliated epithelial cells in the bronchi and bronchioles, and it subsequently appeared in macrophages in the connective tissue beneath.

Effects of iron oxide particles on bactericidal activities of the lungs were studied. At four hours following a bacterial challenge (10^5 bacteria per animal), the mice exhibited a depression of bactericidal activity in the lungs. When challenged with S. aureus immediately following iron oxide exposure, there was a 5 to 65 percent decrease in bacterial killing as compared to matched controls. This impairment was reversible, however, in that 24 hours following a three-hour exposure to 300 and 400 mg/m^3 iron oxide aerosol, the animals exhibited normal bactericidal activity as compared to matched controls. More recently, lung lavage was performed on exposed and control mice to examine possible changes in the nature of the macrophage population. Preliminary data suggest that the iron oxide exposed mice have a lower macrophage viability as determined by Trypan blue exclusion.

A method has been developed which permits the estimation of phagocytosis rates in vivo. Syrian golden hamsters received intratracheal injections of a colloidal solution of Au-198 or were exposed to aerosol (mass median aerodynamic diameter, 1.1μ) of aggregated gold colloid. At 0, 2, 4, 6, 8, 10, and 24 hours post-exposure, the hamsters were sacrificed and the trachea was cannulated. The lungs were washed 12 times with saline and the number of cells and gold particles in each wash determined. Initially, the gold washed out exponentially while the cells washed out with a different but characteristic pattern. As time passed, the gold washout curve gradually approximated the macrophage washout curve. Curve-fitting methods, using least-squares techniques, result in a straight line,

suggesting that the uptake rate is directly proportional to the amount of colloid available. Similar results were obtained from the hamsters.

The method was used to determine the influence of inhaled or intratracheally instilled particles on the endocytosis of gold particles. Evidence was obtained for two kinds of effects: (1) competition, and (2) cytotoxicity. In the first instance, there was a reversible competitive inhibition of endocytosis. In the second instance, the first particle species apparently damaged the cell and its phagocytic apparatus and thus impaired endocytosis.

A crucial aspect of many projects relating to aerosol deposition in the lungs relates to ability to measure ventilation (tidal volume and frequency) in unanesthetized, unrestrained animals. This problem has been largely solved by adapting a plethysmographic technique that was first introduced by Drorbaugh, Fenn, and Chapin. This method is based on the assumption that when an animal in a closed chamber inhales, the inspired tidal volume is warmed from chamber temperature to body temperature and saturated with water vapor at body temperature. The reverse process occurs upon expiration whereby the tidal volume is cooled to chamber temperature and loses water vapor to the extent necessitated by the cooling. In a closed plethysmograph, this warming and wetting of inspired gas and the cooling and drying of the expired gas are accompanied by small pressure changes detectable by sensitive pressure transducers. This technique is particularly suited to aerosol studies since it does not require any restraint on the animal. The measured frequency response agrees well with that expected from a theoretical, electrical analog model based on the geometry of the system. Furthermore, comparative measurements indicate that the electrical signal from this plethysmograph accurately mimics tidal volume measurements made with a head-out pressure plethysmograph.

Development of the cilia of the bronchial epithelium has been studied. Ciliary development was found to be acropetal. As the bud elongates it undergoes internal development, beginning at its point of junction with the basal body (centriole) beneath. At first, internal development of the ciliary fiber complex (the 9 plus 2 configuration of the mature cilium) lags behind the elongation of the shaft; but after the cilium has reached from one-fourth to one-third its final length, the internal organization of the shaft becomes essentially mature from base to tip. Thereafter, elongation of the shaft and development of the internal structure proceed more nearly hand-in-hand.

Each of the nine doublet fibers in the ciliary shaft is continuous with a corresponding triplet-fiber in the basal body wall; only in undergoing transition from the basal body to the cilium, one of the triplet-fibers drops out. Internal development of the ciliary shaft proceeds.

Experiments were carried out on the potential migration of Kupfer cells to the lungs. The movement of Kupfer cells labeled with gold was followed. In mice exposed to fly ash or iron oxide, stimulated with progesterone, or subjected to surgical stress, and in mice with intravenously injected carbon, no evidence of the movement of Kupfer cells to the lung was seen. These results are at variance with qualitative histological techniques used in the past.

Origin of Alveolar Macrophages. The origin of alveolar macrophages was investigated by use of mouse irradiation chimeras, in which the marker to identify the cellular origin of individual cells was a single, known antigenic specificity difference between donor bone marrow cells and recipient somatic cells. By using cytotoxic, monospecific antibody to identify donor cells. The origin of alveolar macrophages in mouse irradiation chimeras was determined at selected time intervals after irradiation and bone marrow transplatation.

It was found that at 21 and 28 days after irradiation, 80 percent of the animals had greater than 80 percent replacement of marrow with donor tissue. White blood cell and alveolar macrophage counts approached normal. Prior to 21 days post-irradiation, replacement of recipient animals with donor hematopoietic tissue was less complete and more variable. However, the percents of donor cells in the alveolar macrophages and in the bone marrow closely paralleled each other. This immunologic approach suggests that alveolar macrophages in the normal mouse are entirely of hematopoietic origin.

Cytotoxic tests were performed on alveolar macrophages and bone marrow cells from exposed to iron oxide and unexposed chimeras in order to identify the origin of macrophages. It was concluded that the pool of alveolar macrophages was increased in exposed animals by cells of hematopoietic origin. This is apparent since no significant difference is seen in percent of marrow cells of donor origin as compared with the percent of pulmonary macrophages of donor origin in the expected animals.

A method has been developed to determine phagocytic parameters in small samples of macrophages. This makes possible: (a) rapid screening of cells, (b) ability to concentrate cells and separate them completely from non-associated substrate, and (c) ability to score each macrophage individually and to eliminate particles absorbed to the cell surface. The system is easily adaptable to situations where less than 10^6 cells are available. In spite of AM pool sizes in this range, in three of the six animals reported, it was possible to screen the pools for WBC contamination, test them for viability (Trypan blue exclusion), split them into four incubation groups, and retrieve them in sufficient concentrations so that typically 200 cells could be scored in one side of a hemocytometer chamber.

Inhalation Toxicology Exposure Systems. An exposure chamber and a dust generating system have been developed that offer a system for testing particulate collection devices in a chamber with uniform distribution of aerosols. In another study a method was developed for assessing the smog forming potential of hydrocarbon mixtures in air. This involved procedures for measuring the reactivity of such hydrocarbon mixtures. In addition, much attention was paid to improvements in calibration of gas-sampling values for gas chromatography, and the use of adsorbents for collection of vinyl chloride and NO_2 from the atmosphere.

A new type of personal sampler for toxic gases in the environment has been developed. The device is based on well-established principles of molecular diffusion. The mass transfer of the contaminant (the amount collected)

depends on three constants: the coefficient of diffusion, length of the tube and cross sectional area of the tube and on two variables: concentration of the contaminanant gas and the time of exposure. The sampler does not require pumps or flow regulators and is light, inexpensive and unbreakable. It has been shown experimentally to be applicable to the determination of several atmospheric gases and, theoretically, could be applied to any gas. The nitrogen dioxide samplers have been supplied to a number of governmental agencies, industries and universities for evaluation under both laboratory and field conditions. Where it has been possible to compare results with those obtained by standard procedures, the agreement has been excellent.

Industrial Products, Byproducts and Intermediates

Epidemiological Studies

A number of cohorts are under study and/or surveillance with exposure to a wide variety of industrial organic chemicals.

Garnetting Workers. A clinical survey of the pulmonary status of garnetting workers is underway in garnetting plants in the Hudson-Albany area of New York. These workers are exposed to large amounts of dusts which often include synthetic organic fiber, fillers, cotton, sizings, finishers, etc. The clinical survey includes physical examinations, chest x-ray, pulmonary function tests, and medical and personal histories, including smoking habits, nature of job, and respiratory infections.

Carbon Black and Oil Mists. A retrospective-prospective study of mortality experience of printing pressman exposed through their work to these substances has been completed. Analysis of the data is underway and will provide the first long-term data on mortality-exposure relations in this group.

Gravure Printing. Toxic exposures in the printing industry are varied. In gravure printing, the pressmen are exposed to complex dyes and chromic acid instead of the usual carbon black and oil mists. Approximately 800 individuals employed in gravure printing in plant in Chicago for at least 15 years are being traced with respect to mortality and cause of death.

In mutagenesis screening of dyes used in the printing industry, it was found by use of the S. typhimurium/microsomal test system that two of 18 dyes tested are mutagenic, e.g., para red and dinitroaniline orange. Enzymatically reduced products of these dyes are being examined as possible primary mutagens.

Coal and Related Products. Currently, research projects are continuing on the biological effects of energy related materials and processes. These are coal, petroleum process components, and automotive emissions. This year work was started on the chemical and biological effects of anthracite coal from a Pennsylvania mine.

The continued four-pronged attack on pathogenesis of disease from exposure to coal dust involves (1) a delineation of inorganic and organic components of bituminous coal from two different geographic areas in which the incidence of coal workers' pneumoconiosis differs markedly; (2) an examination of the biochemical characteristics of the coal dust and their soluble components; (3) the development of a cell culture bioassay system to determine levels of toxicity as well as effects on collagen metabolism; and (4) a study of pulmonary responses in terms of defense patterns and localization of respired material in the lung.

It was previously shown that cells in culture can serve as sensitive indicators of the cytotoxicity of different coal samples and chemical fractions of these coals. A variety of in vitro cell systems have been used to detect different cellular responses with coal extracts from Pennsylvania and Utah coal dusts. The Utah extracts seemed to decrease the number of cells in culture but the cells were larger than in the controls. The bituminous Pennsylvania extracts continue to be more toxic than the bituminous Utah coal extracts no matter what cell system is used.

The study now includes coal from an active anthracite mine in Pennsylvania. Micronized samples of the coal were examined with the same methods as the bituminous samples from Pennsylvania and Utah mines. To date, the metallic content of the anthracite has been determined as well as its effect on pulmonary macrophages and cells in culture. The nickel and chromium content was much lower than Pennsylvania bituminous. It contained about one-half the concentration of free silica in comparison with bituminous. A finding that macrophages harvested 24 hours after dust instillation engulfed fewer anthracite than bituminous particles suggests that deposition of anthracite in the lung may be greater because it is removed less effectively than bituminous particles. When examined with the cell culture assay system, anthracite coal inhibited cell growth approximately 30 percent, the Utah bituminous inhibited cell growth approximately 40 percent, while the Pennsylvania bituminous inhibited cell growth 80 percent.

Previously reported studies on the cytotoxicity of coal dusts used mouse L-cells as a test system. A human cell line from embryo lungs has now been exposed. Significant effects of coal extracts were noted on collagen synthesis as measured by hydroxyproline concentration per unit of DNA. Extracts of different samples have been further fractionated into a variety of neutral, basic and acidic constituents. Activity of these fractions was measured by their ability to inhibit galactose oxidase (a metalloenzyme) and glucose oxidase (an enzyme not requiring metals). On

the basis of a limited number of comparisons, the metalloenzyme proved more sensitive to inhibition than did glucose oxidase.

Organohalides

1. PCBs. Aroclor 1254 (PCB) administered in the diet caused moderate to severe vacuolar degeneration of periportal hepatocytes, hepatocyte enlargement, lipid accumulation and necrosis of the liver in rats. The conversion of acetate to fatty acids was decreased 43 percent by Aroclor, and citrate cleavage enzyme was inhibited. There was no significant change in the distribution of radioactivity in the total phospholipids and triglycerides by PCB *in vitro*, when either glycerol-3-phosphate (uniformly labeled ^{14}C) or ^{14}C-palmitate were used as substrates.

   Four groups of sexually mature male chickens (four per treatment) confined to individual cages, were fed a corn-soybean meal diet containing 0, 10, 20, and 40 ppm PCB (Aroclor 1254), for a period of 40 weeks to observe long-term effects of PCB intake on reproductive performance. The results of this experiment indicated that a 40 ppm PCB intake caused significant reduction of semen volume as compared to that of males on the control ration. No significant differences were observed for semen concentration, fertility, hatchability of fertile eggs, body weights and feed consumption between treatments. Further, fertility and hatchability were not affected when eggs were taken from hens 10-17 days after insemination. Excepting one male which died in the 40 ppm group (diagnosed as tumor in the bursa of Fabricius), all of the birds appeared to be normal and healthy throughout the observation period.

   Studies on metabolism of polychlorinated biphenyls (PCB) in rats concentrated on the development of better methods of extraction and separation of parent compound and various metabolites in both urine and feces. The methods depend on acid hydrolysis, solvent extraction and gas-liquid chromatography using a flame ionization detector. Only 5 to 10 percent of the PCB metabolites excreted in urine are extracted into ether in the absence of acid hydrolysis. Hydrolysis of fecal material does not increase the yield of ether-soluble products. The fecal route does appear to be the major route of excretion of both parent isomers and metabolites of Aroclor 1016. In parallel with these studies, metabolism and excretion of PCB is also under

investigation in a small number of Rhesus monkeys. In another study, the effects of Aroclor 1242 via topical application, as well as that of other compounds, on sebaceous glands of hamsters are being evaluated with the electron microscope. In collaboration with scientists at the Oregon Regional Primate Center, the effects of Aroclor 1242 on the skin, gastric mucosa and liver are being studied with electron microscopy.

The use of cultured cells for PCB studies has now been supplemented with an organ culture technique. This system is particularly useful in studies on teratogenesis. It was found that Arochlor 1254 inhibits development of embryonic chick limbs grafted on the chick chorioallantoic membrane, with little effect on the host embryo. In contrast, Cd proved more cytotoxic than teratogenic in this system. Recent studies utilized in vitro culture of embryonic mouse limbs. The model was developed by comparing the effect of cytosine arabinoside (Ara-C), which causes limb malformation after administering to the whole animal, to the effects after in vitro exposure. The malformation effects in vitro are morphologically similar to those observed through in vivo exposure. A study of a series of compounds is in progress. The technique clearly possesses great potential. Similar experiments have also been carried out using the regenerating salamander limb as a model for detecting or assessing teratogenic effects.

2. Antibacterials. The membrane of the human erythrocyte was used as a model to investigate the influence of halogenated antibacterials on membrane structure and function. Hemolysis in isotonic solutions, altered membrane permeability, and stabilization against hypotonic hemolysis resulting from exposure of erythrocytes to halogenated antibacterials were measured and served as criteria of membrane related effects. The hemolytic potency of the compounds studied differed widely; the observed order of effectiveness was hexachlorophene (HCP) > 3,5-tetrachlorophene (3,5-TCP) = trachlorocarbanilide (TCC) > 3,4-tetrachlorophene (3,4-TCP) > 4,6-tetrachlorophene (r,6-TCP) > dichlorophene (DCP) > tetrachlorosalicylanilide (TCS) = tribromosalicylanilide (TBS).

Measurement of the efflux of K^+ from erythrocytes showed that each of the antibacterials alter the monovalent cation permeability of the membrane. As with other antibacterial-medicated membrane effects, the magnitude of the effect on membrane premeability differed widely among the antibacterials. The

observed effects on first order rate constants for K^+ efflux were HCP > 3,5-TCP > 4,6-TCP ~ 3,4-TCP > DCP ~ TCS > TBS > PCP.

Studies have continued on the biochemical effects of hexachlorophene (HCP) and other halogenated antibacterial compounds on the central nervous system. Rat pups repeatedly dosed with HCP show decreased amounts of brain myelin. This is accompanied by increased activities of the enzyme 2,2'-cyclic AMP phosphohydrolase (CNPase).

Beef rain synaptosomal Na^+, K^+-ATPase was inhibited by very low concentrations of the halogenated antibacterials. The I_{50} values ranged from 0.42 μmoles/mg protein for HCP to no inhibition at 5×10^{-5} M for 3,4,4'-trichlorocarbanilide. Dephosphorylation of p-nitrophenol phosphate (PNPase) by synaposomal preparations was also inhibited but at somewhat higher concentrations of the halogenated compounds. These results are consistent with an inhibition of both the Na^+-stimulated phosphorylation and the K^+-stimulated dephosphorylation steps of the ATPase reaction.

3. Hydrazine. Hydrazine has been found to cause increases (often exceeding 30-fold) in ornithine decarboxylase (ODC) activity. Various substituted hydrazines caused lesser increases. Of these, 1,2-disubstituted compounds were more effective than those with one substitution, and the least effective was 1,1-dimethylhydrazine. No inhibition of ODC activity was caused by 0.5 LD_{50} doses of the hydrazines. Hydrazine doubles the inactivation half-time of ODC, but it is not established whether this change is entirely responsible for the increased activity.

   Hydrazine (0.5 LD_{50}) causes an inconsistent effect on S-adenosylmethionine decarboxylase, probably due to the great variability of the activity of the enzyme.

   Effects of hydrazines on DAO and on oxidation of selected amines are being measured in males and pregnant females. These systems are extremely sensitive and minimum effective dose rates are still being established.

4. Dermal sensitizers. Cutaneous sensitization by three commonly used aromatic aldehydes and inhibition by specific agents are under study. These include citral, which is inhibited by d-limonene; cinnamic aldehyde,

which is inhibited by eugenol; and phenylacetaldehyde, which is inhibited by phenylethyl alcohol. The four levels of biological process at which inhibition may occur include: (1) percutaneous absorption; (2) conjugation with proteins in the formation of active antigens; (3) transfer of antigen by macrophages to lymphocytes and lymphocyte (T cell) membrane activity; and (4) response of sensitized T cells to challenge.

A study of the effect of the inhibitory agent on percutaneous absorption of the sensitizer has been started. Most of the work in the past year addressed itself to possible interference with protein conjugates. Aldehydes generally react with terminal amino groups to give rise to Schiff bases. The quenching effect of compounds like eugenol in the case of cinnamic aldehyde appear to prevent the formation of the Schiff base by extensive hydrogen bonding with the amino group. Phenylethyl alcohol may quench the sensitization of phenylacetaldehyde both by hydrogen bonding to protein side chains and by acetal formation with the aldehyde. A guinea pig model is being developed to examine competitive aspects of inhibitors at the induction and challenge level and to provide cells for in vitro testing of lymphocytes and macrophages.

Allergic contact sensitization to methylene bis(4-cyclohexyl) isocyanate, a substance not previously known to sensitize skin, was found to be the cause of dermatitis in two polyurethane molders. No respiratory sensitization was found in polyurethane foam operations but symptoms induced by a polyamine catalyst (N-methyldicyclohexylamine) were reported by several workers. No threshold limit value or other health standard currently exists for this compound. Phototoxic dermatitis was also observed in a group of roofing workers exposed to various kinds of pitch. The potential toxicity of these types of pitch is now under investigation.

The fact that skin erythema is an important endpoint in many studies of skin toxicology led to a detailed investigation of components of this phenomenon. A skin window technique was further developed permitting direct visualization of the subepidermal vascular plexus during or following manipulation of the overlying epidermis. Suitable chambers for this purpose have been successfully implanted and maintained in the dorsal skin of mice and rabbits. Experiments with guinea pigs were less successful. Initial studies have shown that erythema consists of changes in one or more components including arteriolar and venular diameter, increase in capillary flow, capillary dilation or stasis. Work is proceeding to define the physiologic, pharmacologic and toxicologic basis of these changes.

Carcinogenicity and Mutagenicity of Industrial Compounds. Studies on the sites of interaction of β-propiolactone (BPL) with tissue components indicate that BPL binds in vitro to DNA, histones and non-histone chromosomal proteins in mouse skin chromatin and that among the histone classes, the lysine-rich histones H1 and $H1^0$ bind the most BPL. In order to determine if in vitro reaction of BPL with histones results in binding of BPL to L-lysine, the model compounds ε-N-(2-carboxyethyl)lysine (CEL)

from BPL and L-lysine were synthesized. BPL was reacted in vitro with calf thymus histones and, following proteolytic digestion of the BPL-reacted histones and cation-exchange chromatography, BPL but not CEL was identified as a product of the in vitro reaction between BPL and calf thymus histones.

In studies to determine if BPL reacts with phosphate groups in DNA to form phosphotriesters, model experiments were conducted in which BPL was reacted with thymidine-5'-monophosphoric acid at pH 1.8. The reaction yielded a phosphate ester, designated carboxyethyl ester of Thd-5'-P, whose structure was assigned on the basis of ultraviolet spectra and enzymatic hydrolysis studies.

Preliminary evidence from studies on the in vitro reaction between BPL and calf thymus DNA suggest the possibility that BPL binds to adenine in addition to guanine. In vitro reactions between BPL and mouse DNA or calf thymus DNA resulted in the isolation of a new fluorescent compound which was shown to contain BPL from experiments using BPL-^{14}C. Studies to determine the structure of the fluorescent compound are underway.

Studies on the metabolism of trichloroethylene (TCE), a carcinogen which causes hepatocellular carcinoma in B6C3F1 hybrid mice show that TCE is metabolized in vitro by rat liver microsomes in the presence of an NADPH regenerating system. The reactive intermediate(s) obtained was found to bind covalently to microsomal proteins. Experiments designed to pin-point the chemical nature of the reactive metabolite indicated that TCE epoxide, a highly electrophilic species, is most likely formed, and subsequently binds to cellular macromolecules. These findings support the view that the carcinogenic activity of TCE occurs through the intermediacy of a highly reactive metabolite of TCE, i.e., TCE epoxide.

Since it is known that the incidence of hepatocellular carcinoma is greater in male than in female B6C3F1 hybrid mice, a systematic study pertaining to organ specificity with respect to TCE metabolism in male and female hybrid mice was initiated. In vitro experiments were conducted with microsomes isolated from the stomach, lung, liver, and kidney. Preliminary results indicate that the amount of TCE covalently bound to microsomal proteins increases in the order of kidney, liver, lung, and stomach. The microsomes isolated from organs obtained from male mice were found to bind more TCE than the microsomes from the corresponding organ of female mice. These observed sex-differences with respect to TCE binding correlate with the observed higher incidence of hepatocellular carcinoma in male compared to female hybrid mice.

Results from in vitro experiments using liver microsomes from different strains of rats indicate that the amount of TCE covalently bound to microsomal protein varies from strain to strain and is greater for males than females of the same strain. Microsomes from the livers of Osborne Mendell rats, i.e., animals in which hepatocellular carcinoma cannot be induced by administration of TCE, show a lesser degree of TCE binding after metabolism than microsomes from B6C3F1 hybrid mice.

Investigations were initiated to determine which cellular macromolecules other than microsomal proteins are involved in TCE binding. Preliminary results indicate that a TCE metabolite binds to salmon sperm DNA and that the reactive species involved is most likely TCE epoxide.

A number of flame retardants are being investigated with respect to their chemical stability, reactivity and possible carcinogenic activity. Tetrakis (hydroxymethyl) phosphonium chloride (THPC), a flame retardant widely used on cotton fabrics, was earlier found to decompose to HCl and formaldehyde. In solution, the latter two compounds can form bis(chloromethyl)ether (BCME).

In two-stage carcinogenesis studies on mouse skin, THPC and another flame retardant, Pyroset TKP (the mixed acetate/phosphate salt of the phosphonium ion), were both active as tumor promoters. THPC was inactive as an initiating agent. When tested as a whole carcinogen, THPC gave one squamous carcinoma in a group of 20 ♀ ICR/Ha Swiss mice. Another flame retardant, Tris (2,3-dibromopropyl) phosphate (TRIS) is currently on test for possible tumorigenic activity in mice.

The above phosphonium-based flame retardants were found to react with guanosine, a component of DNA. The product obtained was characterized and identified as a 2-amino substituted phosphine derivative of guanosine.

Investigations of tobacco carcinogenesis with a group of cyclic polyols related to catechol and aliphatic straight chain hydrocarbons show that catechol (1,2-dihydroxybenzene) is a potent cocarcinogen. 3-Allyl-catechol was found to be as potent as catechol, but caffeic acid (3,4-dihydroxycinnamic acid), the allyl catechol compound with a carboxyl group attached to the double bond, had no cocarcinogenic activity. Shikimic acid (3,4,5-trihydroxyl-1-cyclohexene-1-carboxylic acid) and guinic acid were inactive as cocarcinogens. Other phenols present in CSC, e.g., the three isomeric methoxy phenols and o- and m-cresol showed no carcinogenic or cocarcinogenic activity.

Efforts in determining the carcinogenicity of compounds related to vinyl chloride have continued. Vinyl bromide in acetone solution and polyvinyl bromide injections in mice for one year resulted in a large number of sarcomas. These two compounds are also on test in Sprague-Dawley rats using intragastric feeding.

Research on saturated and unsaturated halogenated compounds, which are widely used in industry chemical laboratories and in the environment, has been initiated. The nine compounds selected were vinylidene chloride, tetrachloroethylene, allyl chloride, 1,3-dichloropropene, chloropropene, hexachlorobutadiene, ethylene dichloride, ethylene dibromide and tetrabromoethane. These studies aim to determine the relationship between chemical structure, reactivity with a variety of reagents, stereochemistry, *in vivo* and *in vitro* metabolism and carcinogenic activity.

The first set of experiments carried out concerned the reactivity of these compounds with the important cellular substrate and nucleophile, glutathione.

Allyl chloride was found to be the most reactive compound in this series and vinylidene chloride the least. Interestingly, glutathione was shown to not only displace halogen from saturated carbon, but also to add to double bonds which have halide substituents.

In order to determine the carcinogenic potential of these nine compounds, long-term low dose studies were initiated in rodents using a variety of exposure routes. PMA (phorbol myristate acetate) is used as promoter. Several of the compounds are on test for carcinogenic activity by subcutaneous injection in mice, and a test for carcinogenic activity by subcutaneous injection in mice, and a few are being assayed via intragastric feeding in Sprague-Dawley rats.

Previous studies showed that exposure of purified rat liver plasma membranes to PMA caused a decrease of up to 21 percent of the native membrane fluorescence compared to nontreated membranes. Additional experiments showed that the tryptophan as well as the tyrosine emission of membrane proteins were equally quenched by PMA. The decrease in native membrane emission varied with both the PMA and the membrane concentration. Treatment of rat liver plasma membranes with biologically less active analogs of PMA, phorbolol myristate acetate, a metabolite of PMA on mouse skin, and 4-α-phorbol didecanoate, resulted in a 5 to 10 percent decrease of the native membrane emission. These studies suggest that PMA causes alterations in membrane structure which are due, at least in part, to conformational changes in the membrane proteins.

Studies on the synthesis and characterization of the reactive metabolites of polycyclic aromatic hydrocarbons have led to synthesis of the K-region diepoxide of B(a)P, a bifunctional binding agent which could be an active carcinogenic metabolite of B(a)P. This diepoxide was synthesized via the tetrol and the tetraaldehyde of the parent hydrocarbon. In addition, various new compounds oxidized at both the K-regions and the substituted K-region of B(a)P were synthesized and fully characterized. These B(a)P derivatives will be extremely useful in the identification of similarly oxidized metabolites of B(a)P formed *in vivo* and *in vitro*.

Studies with anthralin (1,8-dihydroxy-9-anthrone) have led to preparation of seventeen structural analogs or potential metabolites of anthralin. The structural features of anthralin necessary for tumor-promoting activity are at least one phenolic hydroxyl group hydrogen-bonded to the C-9 carbonyl oxygen and one benzylic protion at the C-10 position. The results suggest interaction with more than one biological receptor molecule or with a single receptor molecule at more than one site.

Metabolic studies revealed that anthralin is converted to 1,8-dihydroxy-anthraquinone (danthron) and anthralin dimer within five hours of application of anthralin to mouse skin. Further experiments indicated that a factor in mouse skin which is extractable with acetone can almost completely convert anthralin to danthron and anthralin dimer within minutes.

Mechanisms of Carcinogenesis and Mutagenesis. Studies on the use of protease inhibitors as inhibitors of bladder cancer have been continued. N-(4- (5-nitro-2-furyl)-2-thiozoyl]formanide (FANFT) fed to rats resulted in bladder cancer in 12 weeks in all animals. Injection of the protease inhibitors, leupeptin and antipain did not counteract the effect of FANFT. Injection of dexamethasone caused a month delay in onset. Excretion of a protease inhibitor in the urine is increased by adrenal cortical hormones. Thus protease inhibitor may be responsible for the delay in onset of bladder carcinogenesis.

Studies on error prone repair systems for DNA, which result in mutations in bacteria and possibly some cancers in man, show that the induction of this error prone repair system in bacteria is specifically inhibited by protease inhibitors such as antipain. Mutations had been induced in bacteria by ultraviolet light and the induction of the error prone repair system was reduced to less than 10 percent of controls by 10^{-5} M antipain. The effectiveness of the carcinogen, β-propiolactone, as an initiator of two-stage carcinogenesis in mouse skin, was inhibited by substaneous injections of the protease inhibitors antipain and leupeptin. This reduction of tumors in mouse skin may be due to an inhibition of the induction of an error prone repair system in mouse skin as was found in bacteria.

Studies on the possible role of arsenite show that arsenite inhibits steps in postmitotic repair, while chromate appears to inhibit excision repair. The precise mechanism of this surprising diversity of action of these two carcinogenic compounds may involve the inhibition of nucleases responsible for the removal of errors in DNA. Arsenite inhibits the exonuclease V, an enzyme associated with post-nitotic repair in E. coli.

It was found that the tumor promoter, phorbol myristate acetate (PMA) produced a rapid decrease in cyclic adenosine monophosphate (cAMP) in mouse skin. Theophylline inhibited skin cAMP phospodiesterase, raised cAMP levels, prevented the PMA induced cAMP decrease, and inhibited promotion by PMA. PMA reacts with membranes of skin and other tissues to produce effects which are associated with a decrease in cAMP and an increase in cGMP.

Prostaglandins, which are known to increase cAMP levels in cells, inhibit PMA promotion in mouse skin. Studies are continuing on the effects of various tumor promotors and inhibitors (e.g., steroids) on the levels of cAMP and cGMP in mouse skin.

The hemolysis of mouse red blood cells by PMA is a convenient model system for determining the nature of the membrane receptor site for PMA. The hemolysis was specifically inhibited by gangliosides in the order $GM_2 > GT_1 > GD_1 > GM_1$. The molar ratio of interaction was $GM_2/PMA = 1.4$. The hemolysis by anthralin, a promoter of carcinogenesis in mouse skin of moderate activity was not inhibited by gangliosides. This suggests the possibility of different receptor sites for the two promoters.

Mutagenicity studies were carried out on dimethylcarbomoyl chloride (DMCC) and diethylcarbamoyl chloride (DECC) and demonstrated that both compounds are mutagenic in qualitatively similar ways. Since DMCC has been found to be carcinogenic in mouse skin and subcutaneously and to produce squamous cell carcinomas of the nasal passages of rats following inhalation, DECC should be considered a suspect carcinogen.

Further evidence has been found that 2-hydroxyamino-1-naphthol may be the active mutagenic metabolite of the human bladder carcinogen, 2-aminonaphthalene, from mutagenicity studies with various tester strains of yeast, Saccharomyces cerevisiae. Increases in the frequency of petite mutations, mitotic crossing-over and gene conversion have been demonstrated. In other experiments attempts have been made to develop a model system for cell transformation by radiation to study the characteristics of malignant transformation in vitro by radiations and chemical carcinogens in this system, to investigate the interactions between radiations and chemical agents, and to examine the role that cell proliferation plays in these interactions. Studies of the dose-response relationship indicated that the transformation frequency per viable cell rose exponentially with dose for both X-ray and UVL irradiation at doses up to about 400 rads of X-rays and 75 ergs/mm^2 of UVL. At higher radiation doses, the transformation frequency per viable cell leveled off and remained constant at about 10^{-3} following doses up to 1,500 rads or 250 ergs/mm^2. The point of inflection on the dose-transformation frequency curve occurred in the same dose range as that on the dose-survival curve where cell killing became exponential with dose.

The incorporation of the thymidine analogue BUdR into cellular DNA enhanced cellular sensitivity to X-ray induced transformation, implicating DNA as the target molecule in the cell. Splitting the radiation dose into two equal fractions separated by several hours had no influence on the ultimate transformation frequency at a total dose of 150 rads, but led to a decline in transformation frequency at higher total doses (300-800 rads).

Experiments have been performed to study the factors involved in the fixation of the initial transformation damage following x-irradiation. This damage is presumed to represent irreversible mutational changes in cellular DNA. Cells were irradiated in a resting, non-proliferative phase (confluent or density-inhibited cultures), irradiated, then maintained in a non-proliferative state for varying repair intervals from one to 48 hours after irradiation before subculture at low density to assay for transformation. A biphasic response has been observed. With repair intervals of two to four hours a marked enhancement in the transformation frequency occurred over that observed with no repair time or when the cells were irradiated during exponential growth. This is the repair interval which also yields the maximum increase in survival. With repair intervals of 12 to 48 hours, the transformation frequency was markedly reduced. Preliminary experiments have shown that one cell division must occur within 24 to 48 hours after irradiation to fix the transformed state; otherwise, no transformation will occur. On the basis of these results, it is postulated that two types of repair processes occur: a rapid error-prone process, and a more slowly acting error-correcting process. The error-prone process leads to

restoration of the integrity of DNA allowing enhanced cell survival, but leaving behind DNA errors (mutations) which may eventually lead to transformation. Thus, with the shorter repair times, most of the observed transformants would result from these errors induced by the repair process itself. Such error-prone repair processes are known to be an important mechanism for mutation production in bacteria. Their general importance in mammalian cells, however, remains an open question.

When cells were irradiated at increasing densities (progressively fewer cell divisions possible before the cultures became confluent and proliferation ceased), the transformation frequency declined sharply, falling to zero when more than 400-800 cells were plated (comparable to 12 to 13 cell divisions). This suggests that about 12 cell divisions are necessary after fixation in order to express the transformation state; the validity of this interpretation is currently under investigation. Significant synergistic interactions between X-rays and benzo(a)pyrene (BP) have been demonstrated following sequential treatment of exponentially growing cells. Dose-dependent malignant transformation has been obtained with BP alone over the dose range of 1.2 to 6.0 μg/ml medium. When 2.5 μg/ml of BP and 400 rads of X-rays were administered together, a peak enhancement in transformation of five to six fold (over that expected if the effects were additive only) occurred when treatment with either agent was followed 18 to 24 hours later by exposure to the other agent.

Efforts have been made to identify differences in the X-ray response of stationary but potentially proliferative cells as compared with that of cells in rapid exponential growth, as these may be of value in predicting the response of such cell populations *in vivo*. Of particular interest has been the demonstration that stationary cells will repair potentially lethal radiation damage if allowed to remain in a non-proliferative growth phase for several hours after irradiation, leading to a marked enhancement in survival. This enhancement in survival, however, is associated with an increase in the transformation frequency per viable cell. Incubation with DNP (dinitrophenol) during irradiation markedly enhances survival of stationary cells, and enhancement was seen following irradiation with X-rays at dose-rate gamma irradiation (95 rads/sec).

The effect was evident in four aneuploid cell lines, but was completely absent in two non-transformed ("contact inhibited") cell strains. Exposure to DNP under identical conditions (time and concentration) had no influence on survival following Cobalt-60 gamma irradiation delivered at high dose-rates (90-220 rads/sec). The cells were still capable, however, of repairing damage following irradiation at this dose-rate. Several other inhibitors of oxidative metabolism have been tested for their ability to enhance survival in irradiated plateau phase cells. This ability appears to be limited to uncouplers of oxidative phosphorylation; m-chloro carbonyl cyanide phenylhydrazone (CCCP) and dibromophenol were effective, whereas cyanide and sodium arsenates yielded no enhancement in survival. Detailed studies with CCCP yielded similar results as did DNP; these agents also appear to inhibit the rejoining of X-ray induced DNA strand breaks.

Studies of Genetic Susceptibility to Cancer in Human Populations. It has been hypothesized that certain groups in the general populations may be particularly sensitive to the mutational or carcinogenic effect of radiation and other environmental agents. One possible cause for such increased sensitivity could be deficiency in the normal cellular DNA repair processes. Thus, attempts have been made to identify such sub-populations and to determine the relationship between their sensitivity to X-ray induced cell killing and mutations, and their DNA repair capacity.

Cells have been examined from six patients with the progeria syndrome. In two of these strains, a marked reduction was found in the rate at which DNA strand breaks were rejoined following irradiation. Both of these strains had a very limited lifespan in vitro (11 and 17 mean population doublings respectively). When one of these strains was transformed with SV-40 virus, normal repair capacity returned. The rate of repair in normal human diploid cells was found to decline with age, but only in the last few generations before terminal senescence and death of the cultures. In some, these findings suggest that this approach may not be a fruitful one for studying mechanisms of radiosensitivity, though it may be of considerable interest in the study of mechanisms of aging.

A second study involved Fanconi and Downs cells, which normally have chromosome abnormalities or instabilities inherently more sensitive to radiation, or perhaps deficient in DNA repair capacity. Cells from three Fanconi patients and two Downs patients have been examined for X-ray and UVL survival and for post-irradiation DNA strand rejoining capacity. X-ray and UVL survival curves were within the range of normal.

Cells from patients exhibiting usually severe skin reactions to radiation were studied on the hypothesis that a severe reaction may carry a mutation for increased radiosensitivity of cells. Cells were from four such patients and their in vitro survival parameters were compared with cell strains isolated from two normal individuals and one patient who showed an unusually mild response to high total dose irradiation. No clear-cut relationship between clinical responses and intrinsic cellular radiosensitivity was found.

Human tumor cells were studied also to determine whether tumor curability in vivo is related to the intrinsic radiosensitivity of its cells. Tumor cell lines were isolated and propagated in culture from two human osteogenic sarcomas, a human glioblastoma (tumors relatively incurable by radiation) and from two human medulloblastomas (relatively radiocurable tumors). Single dose X-ray survival curves showed no significant differences among the cells. The D_0s ranged from 130 to 144 rads, similar to those of normal skin fibroblasts.

The question as to whether the decrease in the rate of apparent rejoining of DNA between 1.0 and 2.5 minutes results from an actual decrease in ligation, or an increase in incision, can be answered by performing similar experiments in hybrid, radioresistant cells. Rapid repair occurs in the hybrid cell HD_1 and one of its present lines H_4. Resistant cells demonstrate more complete rejoining at any given time, and probably results

from an incisional process which occurs in a cyclic or periodic fashion. It is clear in the case of the HD_1 cells that there is a dramatic reduction in molecular weight during the period from 1.0 to ~2.5 minutes.

It was observed that synchronized V79 Chinese hamster cells, if irradiated at different phases of the intermitotic cycle, evidence incisional repair processes at only one phase of the cell cycle G_2. Cells in G_1 or S phase appear not to perform this incisional process. The duration for which DNA is observed in lower weight species is 1.0 to 1.5 hours. The initial incisional process appears to reduce DNA size to ~29S.

The *in vitro* model of cellular senescence of Hayflick (1965) suggests that cells which senesce in culture reflect an aging process related to that *in vivo*. The growth potential of cell cultures derived from minced whole embryos of the Syrian golden hamster have been measured. Two general patterns of growth potential can be observed in such cultures, depending on the conditions of cell culture.

Further, DNA repair by two techniques for cells which follow each of these patterns have been measured; i.e., unscheduled DNA synthesis (UDS) and rejoining of γ-ray induced strand breaks. For cells which assume exponential growth, UDS is observed at substantial levels after both γ-rays and ultraviolet light (UVL). For cells which senesce, the pattern is related to growth potential. UDS occurs until the final senescence. On the other hand, the rejoining of single-strand DNA breaks shows more sensitivity to the cells' senescing process.

Repair of endonuclease sensitive sites has been studied for endonuclease sensitive sites in cellular DNA *in situ*. *M. leuteus* extracts have been shown to possess endonuclease activities toward both UV- and γ-irradiated DNA. A method of permeabilizing the membrane of mammalian cells in culture was developed that allows such endonucleases to act upon chromatin within the nucleus of irradiated cells. Breaks produced by action of these endonucleases are then assayed on alkaline sucrose gradients. In HeLa cells, endonuclease susceptible sites are removed during post-irradiation incubation. The results of site-removal experiments for a number of other cell lines have been performed and it was shown that XP (type A) and heart endothelial cells repair more slowly or to a lesser extent than HeLa, LICH and NI220 (normal human skin fibroblast) cell lines.

Numerous studies of synergism between agents in a single cell system have been carried out using a relatively sensitive cell line and a resistant cell line. Initial studies utilized X-rays and UVL as agents which produce different DNA lesions and induce different cell recovery mechanisms, and two drugs which inhibit RNA synthesis but have different mechanisms of action. Actinomycin D represents compounds that intercalate into cellular DNA and produce a steric hindrance for DNA maintenance and repair systems as well as RNA transcription by physically interfering with the enzymes required. Cordycepin-(3' deoxyadenosine), an adenosine analog, inhibits RNA synthesis by being incorporated into RNA in place of adenosine with the OH deficiency at the 3' position preventing further synthesis.

Cordycepin was investigated utilizing asynchronous populations of rat hepatoma cells (H_4) in log phase growth. Cordycepin kills cells in a simple exponential fashion. Patterns of X-ray survival of cells treated with Cordycepin were dependent on drug concentration with the predominant effect being to increase the slope of the X-ray survival curve. The increased sensitivity of cells exposed to Cordycepin to subsequent X-ray treatment persisted for greater than four hours after drug administration. Although immediate Cordycepin post-treatment of X-irradiated cells was less effective than pretreatment, the interaction was still significant. Cordycepin treatment did not appear to reduce split-dose recovery or to inhibit rejoining of single stranded breaks.

The potentiation of cell killing was measured in both cell lines when X-rays and UVL were used in conjunction with Actinomycin D and Cordycepin. X-rays and Actinomycin D and Cordycepin potentiate damage in both cell lines. There is no potentiation in either cell line of the killing effect of UVL when used with either drug. It is concluded that Cordycepin and Actinomycin D act in the same manner as do X-rays and potentiate the effect of X-rays.

Other studies on mutagenic and carcinogenic mechanisms have been conducted with various known mutagens and carcinogens, including nitrosamines, aflatoxins, and benzo(a)pyrene.

The development of a forward mutation assay using S. typhimurium is a direct extension of a selective system, 8-azaguanine resistance, successfully used in mammalian cell, Aspergillus and Neurospora mutation studies. However trivial this development may seem, it affords the ability to measure mutagenic potential of a chemical in the broadest molecular sense. All of the six possible base-pair mutations, a wide range of the possible frameshift mutations and all large deletion mutations will be detected in this form of forward mutation assay in contrast to the "false negatives" which arise as a result of inactivity of a base-pair mutagen in reverting tester strains carrying frameshift mutations and vice versa. More subtle molecular specificity apparently results in 9-aminoacridine appearing as a strong mutagen in one tester strain carrying a frameshift mutation (TA1537) and a non-mutagen in another strain carrying a frameshift mutation in a dissimilar DNA region (TA1538).

Linear concentration dependence was obtained for benzo(a)pyrene and aflatoxin B_1, both frameshift mutagens (reverting TA98) and both believed to be metabolized to an epoxide mutagenic product. Aflatoxin B_1 was found to be a much stronger mutagen than BP. It is interesting to note that aflatoxin B_1 is an extremely potent liver carcinogen, while BP is not.

Another polycyclic hydrocarbon, 7,12-dimethyl benzanthracene, showed interesting kinetics, with a downward sloping dose response beyond 14 µg/ml. Under identical treatment conditions DMBA showed a 20-fold lower revertant fraction than benzo(a)pyrene.

Dimethylnitrosamine, which is not found mutagenic in the Ames standard plate assay, displayed linear response at low concentrations. In this

experiment TA1535, the strain sensitive to base-pair substitution was treated for one hour.

Two compounds found carcinogenic in experimental animals, dimethylhydrazine and ethyl carbamate (urethane), were found to be non-mutagenic after a two hour treatment of TA1535 with 10 percent PMS. In both experiment the concentration of the compound being tested was increased until significant toxicity was observed (56 percent survival at 5 mg-urethane/ml dose level and 4.6 percent survival at 5 mg-dimethylhydrazine/ml dose level).

It has been shown that neoplastic growth does not begin until around day 49. Since cultures were confluent by day 14, this shows that neoplastically potent cells remained in a quiescent state for at least 35 days before resuming division. Also interesting was the finding that when cells were replated at low density as late as day 49, it still required about 35 days post confluence for appearance of transformed foci.

In other experiments, benzo(a)pyrene treated cells were transferred after 10 generations of cell growth had occurred post treatment and the cell density at passage was varied over a wide range. This protocol produced virtually the same dependence of appearance of foci on cell density as was seen at the time of treatment. Extrapolation to 0 density indicated that all cells could give rise to transformed descendants.

Studies are being conducted on carcinogen induced modifications in nuclear chromosomal proteins. Nuclear chromosomal proteins were extracted from chromatin derived from sucrose-purified nuclei from the livers of control male Fischer rats and from hepatocellular carcinomas induced in rat livers after feeding aflatoxin B_1 for nine to ten months (2 ppm in the diet).

Isoelectric focusing of the solubilized protein fractions was performed in tubes in the first dimension and SDS-polyacrylamide gel electrophoresis was carried out on slabs in the second dimension. Molecular weights and isoelectric points were determined for each of the stained protein spots and two-dimensional protein "maps" were plotted from each electrophoretic run.

The two-dimensional electrophoretic distribution of nuclear protein subunits from normal rat liver displayed over 200 resolved components ranging from 15,000 daltons to 160,000 daltons in molecular weight and isoelectric points (pI) ranging from 4.5 to 7.5 pH units.

In other experiments male Fischer rats (75-100 g) were bilaterally adrenalectomized and treated five days later with a sub-lethal dose (approximately one-third LD_{50}) of the carcinogens aflatoxin B_1, diethylnitrosamine, dimethylnitrosamine, thioacetamide, 3'methyl-4-dimethylaminoazobenzene, 4-dimethylaminoazobenzene and 4-methylcholanthrene.

The DNA intercalating agent, actinomycin D, the hepatotoxic but non-carcinogenic analgesic acetaminophen, and the weakly toxic aflatoxin B_1 cognate aflatoxin B_2, were also studied. Cytosol and nuclear fractions were prepared from rat livers. Cytosol receptors were complexed with

(^{3}H) dexamethasone, activated and quantitated, and the binding of glucocorticoid receptors to liver nuclei was determined. The apparent concentration of nuclear glucocorticoid acceptor sites and K_d values were estimated from Scatchard plots fitted by the method of least squares from the experimental data. Because of widely differing solubilities, saline was used as the vehicle for actinomycin D, steroid suspending vehicle was utilized for 3'Me-DAB and 4-DAB, and DMSO was employed for the remaining compounds. No differences in the concentration of nuclear binding sites (n) was noted among these three vehicles saline appeared slightly elevated over the values obtained with DMSO and SSV. The control values obtained for the number of high affinity nuclear binding sites in these studies agree well with values reported by other investigators. The calculated number of glucocorticoid-receptor binding sites per hepatoma tissue culture cell nucleus is 35,000 whereas 33,000 acceptor sites have been found per Fischer rat hepatocyte nucleus. The equilibrium constant of dissociation for these two tissues is of the same order of magnitude. A K_d value of 2.1×10^{-10} M has been reported for hepatoma cells (Higgins et al., 1973), compared to a K_d of approximately 8×10^{-10} M for rat liver nuclei.

Without exception, all hepatocarcinogens (AFB_1, DMN, DEN, TAA, 3'Me-DAB and 4-DAB) significantly reduced the value for the concentration of nuclear binding sites by 30 to 50 percent and the K_d values by 25 to 40 percent. In addition, the skin and mammary gland (but not liver) carcinogen, 3-MC, also reduced values for n and K_d by approximately 40 percent. The hepatotoxic, but non-carcinogenic analgesis AAP, had no effect on n or K_d, whereas the transcriptional inhibitor ACT D (also hepatotoxic but non-carcinogenic) significantly reduced the n value by 16 percent but was without effect on the K_d. AFB_2, a very weakly toxic and carcinogenic congener of AFB_1, has no demonstrable effect on nuclear glucocorticoid binding kinetics.

The *in vitro* binding of AFB_1 by rat liver microsomes resulted in a level of modification equal to one AFB_1 moiety per 60 DNA nucleotides, a level similar to maximum values obtained with this carcinogen in other systems. Substitution of microsomes heated at 100° C for three minutes resulted in no significant binding of AFB_1 to DNA. Repeated precipitation of the DNA from solution failed to remove significant quantities of radioactivity. Treatment of unadducted DNA with formic acid at 25° C was found to release 66 percent of the constituent purine bases, whereas the adduct (I) was almost completely liberated from modified DNA, presumably because of destabilization of the glycosidic bond, a property characteristic of 7-substituted guanine deoxyribonucleosides. Analytical HPLC revealed that over 90 percent of the carcinogen radioactivity incorporated into DNA eluted in the adduct peak, indicating that the compound identified is quantitatively the major product of the reaction between the ultimate carcinogen metabolite and the nucleic acid *in vitro*. Approximately 5 mg of white crystals were obtained from 340 mg DNA, and these were pure with respect to the other 254 and 365 nm-absorbing components by analytical HPLC.

The adduct identified in this study as 2,3-dihydro-2-(guan-N^7-yl)-3-hydroxyaflatoxin B_1 represents the major product formed by the interaction of metabolically activated AFB_1 with guanine of DNA *in vitro*. Spectral and chemical evidence indicates that the adduct bond links N^7 of guanine with C-2 of a 2,3-dihydro-3-hydroxy-AFB_1 moiety. The nature of the linkage was established by NMR and chemical methylation experiments and is further suggested by the facility with which the adduct is removed from DNA in acid, because the glycosidic bond is characteristically weak in 7-substituted guanine deoxyribonucleosides. The guanine and hydroxyl functions process a *trans* configuration, with the absolute stereochemistry established by NMR spectroscopy.

The adduct structure is consistent with the expected product obtained by the reaction of a putative AFB_1 epoxide with guanine residues in DNA. This work extends that of other workers in supporting the hypothesis that the proposed AFB_1-2,3-oxide is quantitatively important as an intermediate in the binding of the carcinogen to nucleic acids. Other carcinogens for which epoxide-derived nucleic acid component adducts have been identified include benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene.

Heavy Metals

Epidemiological Studies

Lead. A battery of neurobehavioral tests for clinical field surveys among some 500 lead-exposed individuals is being developed. Tests include examination of motor function and sensory-motor coordination (Romberg, stepping test, spontaneous eye movements, optokinetic nystagmus, subjective effort, termor) and sensory functions (double simultaneous stimulation, color perception, critical frequency fusion, visucal fixation with passive head movements), perception (hidden figures, large-small characters), spatial vision (block assembly), recent memory (learning and recall), and high functions (picture arrangement, calculations, number order reversal, digit span, digit reversal and word reversal).

As an adjunct to these studies, monkeys are being used to detect early neurobiological and biochemical changes since they afford the opportunity to obtain information about changes over time. Examinations will include cardiovascular and central nervous system functions, behavior, biochemical analyses, renal function, and organ pathology (via autopsy material).

Mercury. Previous reports from Minamata and from New Mexico, indicating that prenatal exposure to methylmercury produced severe damage to the central nervous system, were confirmed in the first reports from the Iraq outbreak. Certain differences were noted from description of the Japanese cases. Blindness occurred in the Iraq babies but was not reported in Japan. In Iraq, if the infant was badly poisoned, so was the mother. However, the Iraqi observations were based on studies in the first few months after the outbreak. Extensive follow-up studies are needed to

more precisely determine the dose-response relation for pre- and early postnatal exposure. The biological half-time in blood and hair were identical for the infant-mother pair in which the infant was prenatally exposed and averaged 58 days. In postnatal exposure the biological half-time extimated from blood samples collected from the infant was significantly greater than the biological half-time in the mother (56 days versus 39 days). This is believed to be due to continued ingestion of methylmercury from maternal milk by the infant.

The mothers of both infants have signs and symptoms of methylmercury poisoning. From the hair levels it was estimated that the maximum concentration of mercury attained in blood was in the range of 1,500 to 3,000 ng Hg/ml. Both these concentrations are extremely high and are in the range that one would expect to find the signs and symptoms according to the dose-response relationship published by Bakir et al. (1973).

The maximum estimated blood level in the postnatally exposed infant was approximately 1,000 ng Hg/ml. Except for moderately increased deep tendon reflexes, no signs of poison was observed in this infant throughout the hospital clinical follow-up period, extending to approximately ten months of age and at thirty-six months of age when she was last examined.

A survey was made of residents of the White Dog and Grassy Narrows Reserves in Canada to determine the extent of exposure to mercury and if such exposure was causing adverse health effects. A mobile trailer equipped for analysis of mercury, was established in the White Dog Reserve in August 1975.

A total of 587 hair samples and 158 blood samples were collected on both Reserves by house to house visits in August. The sampling program is continuing to check those individuals not resident on the Reserves in August. Analysis of these samples is continuing in the laboratory at White Dog. The results obtained to December 1975 indicate a wide range of mercury levels--from less that 5.0 ppb to 330 ppb in blood.

Children usually have lower blood levels than their parents but younger children tended to have higher mercury levels than older ones.

The sampling program in White Dog and Grassy Narrows Reserves is not yet complete and all the samples that have been collected have not been analyzed. Nevertheless, even at this early stage of the program, some tentative conclusions may be drawn:

1. Several individuals in both Reserves still have unacceptably high blood levels of methylmercury.

2. Very high exposure to methylmercury continues to be a serious problem with guides working at fishing camps. The highest concentrations of mercury ever reported from White Dog and Grassy Narrows Reserves, approximately 150 ppm in hair equivalent to 500 ppb

in blood, occurred in the summer of 1975 despite the fact that warnings against eating contaminated fish have been in effect for several years and that an intensive health education campaign has been in effect during the past year.

3. Wives of guides have a higher exposure to methylmercury than other females.

4. A matter of most serious concern is the exposure to methylmercury of pregnant females. One case at White Dog Reserve is described where the infant's blood level at birth was 100 ppb. However, samples of maternal and cord blood collected from mainly non-Indian females in the area of Kenora indicate no significant exposure to methylmercury.

In another study, also arising out of the interests in heavy metal toxicology, the interaction between the metals Zn and Pb in humans and the enzyme ALA dehydrogenase was further investigated. As previously reported, Zn can protect the function of ALA dehydrogenase against its diminution by lead. This finding may be important not only because state of nutrition is again seen to be an important factor in assessing the exposure of experimental animals or human populations to Pb, but also because of possible use of Zn as an adjunct in the therapy of lead intoxication. In one case of human plumbism, the effects of $ZnSO_4$ supplementation on EDTA therapy were investigated. The chelator increased urinary excretion of Zn more so than that of Pb, and decreased activity of ALA dehydrogenase. In a patient with hereditary tyrosinemia, increased excretion of ALA in urine was also observed, together with low serum Zn levels and decreased ALA dehydrogenase.

Titanium Oxide. Studies over the past few year have provided the opportunity to analyze the long-term mortality experience of a large cohort of titanium workers. These studies will provide first data on mortality experience with titanium as an environmental hazard. TiO_2 is widely distributed in the environment. It is used in virtually all consumer and industrial paints, as a filler for plastics, paper and many other materials. Knowledge of any long-term toxic effects in lacking, due possibly to lack of critical evaluation.

Lead. Clinical studies in children showed behavioral abnormalities including hyperactivity as a sequela to lead encephalopathy but no discrenible alterations in children with an increased exposure or burden to lead but without encephalopathy. An "Activity Room" in which this form of behavior can be assessed in a quantitative manner is being utilized in studying children, as a potential sophisticated measure of children with an increased lead burden. The use of tryptophan as a provocative test or a pharmacologic probe of perturbed behavior was studied in rats and now in a clinical population of hyperactive children. The addition of tryptophan to the diet appears to have improved some of the hyperactive children.

The entrance of lead into the nervous system is dependent upon the blood-brain-barrier system. In a series of radiolabeled lead (Pb^{210}) studies, the lead rapidly accumulates in the choroid plexus, periventricular tissues, and leptomeninges. However, labeled lead does not remain or concentrate at these sites.

In other studies of pediatric lead poisoning, various statistical procedures were designed and tested for matching chemical elements in high-lead feces with elements associated with the suspected environmental sources of lead. Basically this approach consists of ranking elements, feces vs. sources, and developing rank correlations. Two main problems were encountered: The high non-source contribution of different elements raises the background, and differential intestinal absorption may further obscure existing differences. It was not possible to differentiate clearly in rat feces between paint and soil sources of lead. Attempts are under way now to separate lead-containing particles from fecal matrix by density gradient centrifugation. Success here might allow more unequivocal identification of the source of lead. Sufficient children with elevated blood levels have been identified to permit active continuation of this program.

Investigations have been carried out to correlate work on the data from pathological examinations and analysis of tissues from over 60 autopsied cases. Correlations for lead and cadmium indicate the respiratory tissue burden for lead is related to airborne materials, but the relationship between the concentrations of lead in lung and lymph nodes is obscured by smoking. Smokers also appear to have higher burdens of cadmium in the lungs, lymph node and liver than do non-smokers. The lung lead burdens in New York City residents have decreased significantly during the past six years. The correlation of the average lead burden in human lungs to the average airborne lead previously reported has been improved by reanalysis of tissue homogenates from 1969-70 using a more precise analytical method and by elimination of cases with significant occupational exposures.

Another study continues to deal with the biological significance of some common indices of lead exposure. One such index is the elevated excretion of delta-aminolevulinic acid (ALA) in urine. A major uncertainty in this work arises from the lack of satisfactory techniques for estimation of the normally low levels of ALA circulating in plasma. There is no agreement in the literature, for instance, on the magnitude of ALA clearance in normal subjects; this fact in turn makes it impossible to decide whether increased excretion reflects at least in part inhibition of tubular reabsorption and thus increased clearance of ALA. A new gas chromatographic procedure has been developed. It is possible also now to check the validity of ^{14}C used previously as tracer for ALA which established that at least in the rabbit fractional reabsorption of filtered ALA is very high and is not decreased by an exposure to lead which greatly increases ALA excretion. It was of interest also to find that significant species differences in the clearance of ALA approach those of creatinine.

Drinking Water Contaminants. Cincinnati drinking water as related to any epidemiologic evidence of carcinogenic activity was investigated. An extensive catalogue of potential biases and problems in interpreting

epidemiologic data from death certificates for the period 1950-1964 was prepared. While statistical analysis failed to show any relationship between chemicals found in Cincinnati drinking water and cancer mortality rates, it is clear that this mortality analysis is only a gross look by the Health Department, City of Cincinnati. Work was continued also on nationwide epidemiologic studies of cancers of lung, pancreas and brain.

Laboratory Studies

Lead. Exposure of newborn mice to lead in the milk they receive during weaning from mothers given lead in their drinking water results in a hyperactivity that persists for extended periods of life. These hyperactive mice exhibit no other overt symptoms of lead intoxication. Treatment of hyperactive mice with a number of centrally acting drugs leads to the hypothesis that at a neurochemical level, hyperactivity is an expression of an imbalance in both cholinergic and aminergic neurotransmission. Centrally active cholinergic and anticatecholaminergic drugs alleviate the hyperactivity while anticholinergic and catecholaminergic drugs exacerbate the hyperactivity. In vivo and in vitro studies of acetylcholine release in peripheral and central nervous tissue suggest that the altered pharmacological and behavioral responses are, at least in part, due to a decrease in cholinergic function.

Catecholamine levels and turnover at 15 days of age are especially interesting in the neostriatum, where the high lead acetate dose (75 mg/kg/day) results in decreased dopamine levels and affect the turnover of dopamine. There is also some evidence of a difference in levels and turnover of noradrenaline in the cortex of low dose subjects. Neither high nor low doses of lead affected weight of the brain regions studied (midbrain-brainstem, diencephalon, neostriatum, or cortex).

Studies with Pb-203 have centered on applications in canine subjects preliminary to those in man. Specifically, the pulmonary absorption of different forms of lead and with different amounts of carrier have been measured in dogs following either intrabronchial instillation or inhalation. Ancillary studies of lead distribution and retention following intra-lymphatic (inguinal) and intravenous administration have also been undertaken to assist in the intrepretation of results and for correction of measurements.

Results to date verify that the most significant pulmonary removal mechanism for lead is direct absorption into the blood. Significant amounts of lead enter the blood through the right lymphatic duct, but this is far less important quantitatively during the first hours post-administration. Blocking the mucociliary transport pathway by a cuffed-intratracheal tube has negligible effect on the short-term clearance of lead.

The rate of Pb uptake from the lungs is modified by the chemical form and the amount of carrier but additional data are needed before any generalizations are proposed.

A coordinated study of behavior, brain biogenic amine, and tissue lead involved 30-day old rats given 0, 25, 100, and 200 micrograms of lead per day, per gram of body weight for the first 20 days of life. While there was a correlation between lead-dosage and blood, brain, and bone lead, no changes in brain (five regions) dopamine or norepinephrine or activity were demonstrable. While still in a preliminary stage, other behavioral studies (T-maze, aggressiveness, operant conditioning studies), also reveal no effect of lead. Studies focusing on chronic intoxication, including prenatal effects of lead, indicate a slower rate of growth in animals exposed to 250 ppm and delayed vaginal opening in females exposed to doses as low as 50 ppm. On the other hand, operant conditioning studies in rats have shown no changes. However, pigeons trained to peck for food showed altered behavior prior to overt toxicity. Of two doses studied, 12 and 25 micrograms, the 25 micrograms of lead per day per gram body weight pigeons produced toxic and fatal effects in about 14 days but affected performance within a few days. The lower dose, 12.5 micrograms, produced few, if any, signs of toxicity but performance was affected but returned to normal slowly after the agent was discontinued.

Studies are in progress to determine the effect of neonatal lead exposure on serum and pituitary FSH and LH levels in rats. Results available at this time indicate that lead treatment reduces serum and pituitary FSH from day 15 in female rats, and serum FSH from day 20 in male rats. Suppression of the serum FSH levels in 15 day old females appears to be dose-dependent.

Studies on the distribution of inorganic lead in guinea pig brain have been extended and completed. In summary, guinea pigs pretreated with lead for five days, show a striking concentration of the radiolead (Pb^{210}) in the choroid plexus (70 times the concentration in brain). A high concentration of Pb^{210} has also been found in meninges and ependyma, which is less than that in choroid plexus, but exceeds markedly that appearing in brain. This contrasts with the finding that <u>total</u> (cold) lead levels in the choroid plexus were not detectable (average level in control animals less than 1.2 µg/g; less than 1.5 µg/g in poisoned animals). Pretreatment with intravenous ouabain ($.17 \times 10^{-9}$ mols/kg) reduced significantly ^{210}Pb accumulation by choroid plexus and by brain at 5 and 60 minutes after label injection.

The steady-state blood to CSF transport of ^{210}Pb is being evaluated using cats subjected to ventriculocisternal perfusion. The pilot studies in this work have advanced rapidly. The control animals received .01 µCi/kg of ^{210}Pb i.v. Average CSF formation (K_f) was .005 ml/min. Terminal tissue-to-plasma ^{210}Pb distribution ratios averaged respectively: 83 (4th ventricular CP), 95 (lateral ventricular CP), 23 (hypothalamus), 6 (caudate nucleus), 8 (thalamus), 5 (ectosylvian cortex), 5 (paraventricular tissue (PV)), 2 (cerebellum) and 10 (meninges). Total lead levels in blood averaged <3.4 µg/100 g. Lead levels in PV averaged 0.24 µg/g, and

lead could not be detected in other brain regions, meninges or CP (≤0.05-0.41 μg/g. Three cats received lead carbonate 165 mg q.d. for five days. This resulted in elevated blood lead levels (15 to 155 μg/100 g). The animal with the highest blood lead showed a clinical picture of acute encephalopathy. Average V_f and K_i in these animals were .035, .005 and .002 ± .0005 ml/min. Tissue/plasma ^{210}Pb levels for all tissues were similar to control animals. Total lead levels in hypothalamus, thalamus, cerebellum and meninges averaged .3, .25, .5 and 1.07 μg/g, and lead was not detected in other regions.

These results have confirmed the initial impression that the neural barrier tissues play a significant role in the immediate uptake of tracer amounts of inorganic lead from the systemic circulation both in control and in poisoned animals. The pilot study on steady-state transport suggests that the same applies in that situation. Since the choroid plexus and possibly other components of the barrier tissues are known to be involved in the regulation of solute transport in brain, these results seem highly deserving of further study. The fact that the tissue levels of total lead in the barriers are small, despite the high initial accumulation of label, would suggest that a short term "sink" effect obtains in relation to the barrier accumulation of lead. The perfusion data also indicate that the possible dysfunction of the barrier organs due to this high accumulation of lead may be a selective one since at least one function, formation of intraventricular CSF, is not affected in lead poisoning.

The neonatal rat, exposed to lead in mothers' milk, has become the main test model for determining central nervous effects of the metal in young animals. Considerable preliminary work was required to determine the relationship between lead exposure of lactating rodents and their offspring. A model was developed which, taking into account the daily increase in body weight of the neonate, the calories required to support this growth, and the changing caloric values of milk during lactation, provides an estimate of daily milk consumption by the pups. Coupling this figure to the estimated lead content of the milk then yields an adequate measure of exposure of the pups to lead. It is important to point out that the levels of lead in the maternal diet were chosen to reproduce in the offspring lead levels in the blood comparable to those found in the pediatric population under study (below 80 μg percent), and that growth of the offspring at these levels was unaffected.

The test animal, as described above, is the neonatal rat. Beginning shortly after weaning, the pups are subjected to a battery of behavioral tests. Contrary to previous findings, no difference in activity has been found between pair-fed normal and lead-exposed animals, nor is there an impairment in the acquisition of a complex skill. Significant changes are seen in the amount of aggressive behavior displayed, with exposed animals behaving less aggressively than controls. Similar experiments are now in progress testing the effects of prenatal cadmium exposure on behavior of developing rats. Initial findings have shown a significant suppression of activity in these animals, but the possible influence of differences in growth pattern remains to be determined.

Mercury and Its Compounds. Chronic dosing with methylmercury, 2 mg/kg for five days a week, yields the rather abrupt appearance of symptoms in one to two months. This sudden appearance has been reported in mice and monkeys.

Changes in performance on the fixed consecutive number schedule include reduction in the rate at which pigeons peck, an increase in the variability of the number of responses they make on the counting key and, generally, a shift toward shorter run lengths. Changes in two pigeons by a dosage regiment lasted about a month for Bird 11 and two months for Bird 12. Control dosage with the vehicle had little effect. In both cases some recovery of function was seen after the passage of many months without further methylmercury.

Using a mouse model comparative kinetic studies have been completed of maternal distribution and maternal-fetal transmission of methylmercury (CH_3Hg^+), mercuric chloride (Hg^{++}), and mercury vapor (Hg^0) during a 48-hour period following molar equivalent, nontoxic (0.5 mg/kg), single doses of each form of mercury. Mercury concentrations of fetal and maternal blood, plasma, brain, liver, kidney, and placenta were determined at 1, 4, 8, 24, and 48 hours. Fetal brain mercury concentration was higher than maternal brain concentration at four hours and was three times higher at 24 hours, when maternal and fetal blood (and plasma) concentrations were comparable. These data show that CH_3Hg^+ rapidly crosses the mouse placenta and concentrates in fetal brain. They provide the basis for comparative studies of Hg^{++} and Hg^0 exposure of pregnant animals. Detailed comparisons of maternal and maternal-fetal transmission of CH_3Hg^+ with Hg^{++} and Hg^0 are nearly completed. These studies provide the basis for assessing approaches to protecting the prenatally exposed fetus by reducing transplacental transmission, and for examing approaches to reducing mercury body burden of exposed fetuses and mothers.

It was observed that excretion of CH_3Hg^+ in new born mice is much slower (estimated half-time of excretion, $\sim$200 days) than in adult mice (half-time of excretion, $\sim$7 days). Recent studies have delineated an abrupt prepubertal increase in excretion of mercury in animals pulse-dosed with Hg^{++} as well as with CH_3Hg^+. Details of renal and gastrointestinal excretion patterns have been determined, and this ontogenetric model is being used to probe mechanism of mercury excretion. For example, phenobarbital and chemical agents known to stimulate bile flow and biliary excretion are being used to determine whether mercury excretion can be increased in young animals that are excreting almost none of their body burden each day. The studies will aid in elucidating the mechanisms of CH_3Hg^+ excretion as well as providing clues to possible modes of therapy.

In accordance with the published reports, it was found that the kidney takes up ^{203}Hg rapidly and about half of the injected dose was accumulated in this organ within 48 hours. Of this, nearly 50 percent was associated with metallothionein produced in response to mercury challenge. The concentration of ^{203}Hg in the kidney was depleted after 48 hours, and 19 days later only two-thirds of the isotope remained in the tissue. A

simultaneous reduction in ^{203}Hg content of metallothionein fraction was also observed. However, about half of the tissue ^{203}Hg was associated with metallothionein at all times, indicating that intracellular distribution of mercury was unaffected by the change in concentration.

The rate of biosynthesis of mercury-induced metallothionein was studied by a two hour plus dose of ^{35}S-cystine in rats injected with $HgCl_2$. Maximum incorporation of the ^{35}S label into renal metallothionein occurred eight hours after mercury injection, unlike the four hour period observed previously for cadmium-injected animals. This suggested that the delayed response in the case of mercury was apparently due to the synthesis of new messenger RNA.

To further identify the site of mercury interaction in controlling renal metallothionein biosynthesis, inhibitors of protein synthesis were employed. Cycloheximide was chosen as a ribosomal level inhibitor and actinomycin D as the nuclear level inhibitor of protein synthesis. In mercury-injected rats, actively synthesizing metallothionein, the incorporation of ^{35}S label from cystine was blocked by cycloheximide. Pretreatment of mercury-injected animals with actinomycin D also resulted in inhibition of ^{35}S incorporation into metallothionein. In addition, the intracellular distribution of mercury was altered and a majority of the tissue ^{203}Hg was associated with molecules of molecular weight greater than that of metallothionein.

The above results indicate that mercury regulates the synthesis of renal metallothionein by a nuclear process which probably involves the production of new messenger RNA. This is in contrast to the previously reported results for cadmium, which apparently utilizes a stable messenger RNA to enhance the production of metallothionein in the kidney. In the light of these observations and the physicochemical properties of the cadmium and mercury induced proteins, it appears that the two proteins may be different. However, both proteins seem to be catabolized at a very similar rate; the half-life of mercury-induced protein in the present investigation was found to be 4.4 days.

In other studies animals were injected i.p. with a single dose of ^{203}Hg labeled methylmercury chloride. After sacrifice the brains were removed and homogenized. A 10,000 xg supernatant was prepared and the pattern of mercury-binding proteins examined by gel permeation chromatography on Bio-Gel P10. Two major fractions were observed, one of high molecular weight and the other with a molecular weight of about 3,000 daltons, which is presumably not metallothionein. In the single injections experiment, the brain mercury and the mercury in each of the two peaks increased proportionately over a five day period. The properties of the 3,000 dalton peak material are currently being examined.

Methylmercury is known to affect only specific areas of the central and peripheral nervous systems. These areas are small compared to the total brain. Several attempts have been made to find the biochemical mechanism of methylmercury neurotoxicity. These include studies on subcellular

fractions, slices, and total brain. Both enzyme activities and protein synthesis have been investigated.

Neurons of systemically poisoned rats showed two signs of electrophysiological damage by methylmercury. The increase of potassium conductance in repolarization was blocked, producing a "plateau" in the spike potential, and impulse conductance was blocked in axons. These signs were located in the same spinal neurons that showed cytopatholical changes.

Early morphological changes were observed in the volume of perikarya, in the smooth endoplasmic reticulum, and in the rough endplasmic reticulum. The changes were transient in non-target neurons, and progressed to local calcifying degeneration in target areas.

Tests of protein synthesis in brains of poisoned animals gave contradictory results in that some authors reported an increase while others reported a decrease. The contradiction may be resolved by the hypothesis that different cells in the brain respond to mercurials by increasing or decreasing protein synthesis at different times and to a different extent. Electron microscopic observations supported this idea. Either an increase or a decrease in protein synthesis could, therefore, be found in the same part of a brain after treatment with methylmercury, as a result of differences in tissue fractionation and in the timing of treatments.

The effects of mercuric chloride also were studied on the repeated acquisition of a 4-step position sequence in pigeons. Doses of 1 and 3 mg/kg/day had no effect on the bird's ability to learn the sequence, although after about a week, rates of responding decreased. After 20 days of administration of mercuric chloride, injections were discontinued and the rate of responding gradually recovered over the next 10 days.

These experiments show clearly that operant behavior is a sensitive and stable baseline for studying the effects of toxic agents on behavior. With lead, mercury, cadmium and parathion subtle effects on behavior could be detected upon chronic administration of small doses, long before any signs of gross toxicity could be observed.

Another important discovery in these experiments is that although the effects of the repeated administration of some toxic agents lasts for a long time after the administration of the agent is discontinued, the animals do seem to recover eventually. For example, with some tests the effects of mercuric chloride could be detected for two months after administration was discontinued, but after three months the behavior was normal.

The experiments also show that some tests are better for studying particular toxic agents than are others. For example, isopropanol increased errors under the repeated acquisition procedure, but vanadium and lead did not. All the experiments taken together show that operant behavior is an excellent baseline for detecting and measuring the effects of toxic agents.

A new assay system was developed to measure the mercury-binding capacity of subcellular fractions. Potentiometric measurements with a silver electrode of new design yield titrations accurate to ±26 nmol of methylmercuric chloride or ±8 nmol of mercuric chloride, and linear with cysteine concentrations ranging from 1×10^{-7} M to 8×10^{-4} M.

A mathematical treatment based on the Hill equation was devised to derive the distribution of methylmercury between enzymes and other ligands (relative affinity), and to compute the susceptibility of enzyme to inhibition in terms of the degree of saturation of total ligands by methylmercury. With this approach, brain preparations were shown to be significantly more susceptible than red blood cell preparations to inhibition of ion transport enzymes by methylmercury and by mercuric chloride.

Two mechanisms of action were found for the inhibition of the active transport of calcium by methylmercury and mercuric chloride. Both mercurials blocked the phosphohydrolase component of the "calcium pump," but only Hg^{++} blocked the ionophore component. This interference with the calcium-binding site was shown to be responsible for the selectively greater potency of Hg^{++} in blocking the active transport system.

Other studies have shown that solubilized Ca, Mg-ATPase (S-Ca^{++}-ATPase) or a fragment Ca, Mg-ATPase from sarcoplasmic reticulum increases lipid bilayer membrane conductance several hundred fold. The relative conductance change and the relative permeability elicited by this material show the following sequence:

$$Ba^{++} > Sr^{++} > Mn^{++} > Zn^{++}, Na^{+}, K^{+}, Cs^{+}, Li^{+}, \text{and } Rb^{+}.$$

The divalent cation interactions with the Ca^{++}-ionophore were consistent with their interaction with Ca, Mg-ATPase and with Ca^{++}-pump. This indicates that the Ca^{++}-ionophore may be an integral part of the Ca, Mg-ATPase enzyme and may function as a Ca^{++}-carrier in the overall Ca^{++}-pump of sarcoplasmic reticulum.

Studies have shown physically and chemically that the catalytic function of Ca, Mg-ATPase studies in the 30 K fragment and that the Ca^{++}-ionophoric site resides in the 20 K fragment. Both 30 K and 20 K fragments are part of the 55 K fragment which faces the myoplasm.

Effects of Se on Hg Toxicity. Adult female rats were given orally a toxic or non-toxic dose of $^{203}HgCl_2$ or ^{75}Se-selenite alone, $^{203}HgCl_2$ plus various amounts of ^{75}Se-selenite, non-labeled selenate, seleno-methionine or seleno-cysteine and ^{75}Se-selenite plus phenylmercuric acetate (PMA), or methylmercuric chloride (MMC). Tissue accumulation, subcellular incorporation and soluble protein binding of ^{203}Hg and ^{75}Se in the liver and kidney were studied.

Selenite treatment modified the tissue distribution, subcellular binding and soluble protein binding of ^{203}Hg in the rat. ^{203}Hg content of all tissues with the exception of kidney was higher in the selenite treated rats, and the magnitude of increase was higher with higher dose up to a

Se/Hg ratio = 1. In contrast, the ^{203}Hg content of the kidney was decreased. Other selenium compounds also gave similar effects and the order of effectiveness is as follows: seleno-methionine > seleno-cystine > selenate > selenite. All four selenium compounds increased the incorporation of ^{203}Hg in the crude nuclear and mitochondrial fractions and decreased that in the soluble fraction. Higher dosages resulted in greater alteration. ^{203}Hg binding to liver soluble proteins was altered by selenite and binding was dosage dependent. However, no significant change of Hg binding pattern in the kidney soluble protein due to selenite was observed.

The effect of methylmercury on reproduction and transplacental neural tumor induction in rats fed sodium nitrite and ethylurea was investigated. A dramatic synergistic effect of methylmercury and nitrite-ethylurea on breeding performance was observed. The performance of rats in the experimental group for conception rate, litter size, weanling weight, and survival was only 32 percent, 62 percent, 40 percent, and 22 percent of controls, respectively. All four experimental rats that survived developed tumors or brain necrosis quicker than rats in the control.

Mercury in Marine Animals. The unusual mercury distribution pattern found in marine mammals has in part been duplicated in studies with rats dosed with ^{203}Hg-labeled methylmercury or $^{203}HgCl_2$ and $Na_2{}^{75}SeO_3$. Injection of $HgCl_2$ and Na_2SeO_3 caused a long term accumulation of mercury and selenium in the liver but prevented accumulation in the kidney. Sodium selenite also caused a long term shift in the subcellular distribution of mercury in liver, but not in the kidney. All of these distributions resemble those found in California sea lions.

Mercury in California sea lion liver tissues appears to be associated predominately with particles of about the same density as mitochondria and containing mitochondrial cytochrome oxidase activity. The mercury binding capacity of these particles may partially explain the ability of the animals to tolerate such high levels of mercury. Extensive sampling of the biota was conducted at Cottage Grove Reservoir from June 1975 through January 1976.

Data on mercury accumulation for the spring chinook salmon indicate that two different populations of chinook exist in the system: (1) those reared in the reservior which contain a high level of mercury, and (2) those reared in the tributaries which contain a lower level of mercury.

Stomach analysis was performed on the chinook to determine feeding habits. No difference in feeding habits could be seen between the two age classes. Their efficiency of mercury adsorption from the food ranged from 55.0 to 94.3 percent.

The cutthroat sampled from the reservoir had a high mercury concentration than those sampled from the tributaries.

Mercury levels were also determined for rainbow trout and brown bullheads. The highest level found was 0.412 µg/g in a one year plus old bullhead taken in July.

Cadmium. As previously reported, there is good evidence to support the hypothesis that not all Cd accumulated in the organs of poisoned animals or man exerts the same toxic effects; reference in this regard need only be made to the absence of any correlation between observed lesion and organ levels of the metal. Attempts to obtain direct proof of tissue compartmentation have thus far, however, not succeeded. These experiments were based on the assumption that Cd present in different chemical forms would be likely to exhibit different rates of equilibration with plasma Cd.

Another approach to this problem is the actual study of various Cd compounds present in the tissues of exposed animals. In particular, progress was made in characterizing such compounds in the lungs of rats which had been exposed to CdO dusts. Significant portions of Cd accumulated in lungs under these conditions are bound to low-molecular weight proteins. These resemble metallothionein, although their metal composition differs from that of metallothionein in the kidney. An interesting new finding is that of the presence in the kidneys of these animals of peptides of much lower molecular weight than metallothionein. These compounds bind a significant protion of total tissue Cd.

The function of metallothionein remains in doubt, and several projects deal with this problem. One of the proposed functions of low molecular weight proteins is that of Cd transport from liver to kidney and of its renal excretion. Other than the fact that the substance can be filtered at the glomerulus, little is known of its handling by the kidney. Earlier work has provided some insight into this question through analysis of the renal handling of a model compound, Cd-mercaptoethanol. These studies have now been expanded to include labeled Cd-metallothionein. A detailed quantitative study has provided values for some of the processes involved in renal reabsorption of filtered Cd-metallothionein in normal and Cd-poisoned animals. No Cd-metallothionein secretion could be observed, and the rate of its tubular reabsorption even in poisoned animals is sufficiently high to throw doubt on a possible role of the compound in Cd excretion.

Further insight into mechanisms of Cd intoxication is sought by a new project on the function of pulmonary enzymes induced by exposure to oxidants. The level of these enzymes in various tissues is influenced by nutritional factors; for instance, chronic Cd feeding decreases glutathione peroxidase in rat plasma and testes and it is possible that such changes are related to Cd toxicity.

Many studies have shown that significant interactions occur between Se and heavy metals such as cadmium, mercuric mercury, methylmercury, and silver. It has been suggested that marine mammals are able to detoxify methylmercury by an interaction with selenium. Although a direct interaction between Se and other heavy metals has been postulated the mechanisms by which Se decreases the toxicity and alters the tissue distribution of these elements is not understood.

^{75}Se and ^{109}Cd tracers were used to study the binding of Se and Cd to plasma proteins at various SeO_3^{2-} doses and times up to 24 hours after the simultaneous subcutaneous administration of SeO_3^{2-} and $CdCl_2$ to adult male rats. This treatment markedly increased both Se and Cd plasma levels over that in controls. Gel permeation chromatography of plasma indicated that at all times up to 24 hours Cd and Se were bound in an atomic ratio of approximately 1:1 in 330,000 and 130,000 dalton fractions. From 4 to 24 hours, Cd and Se appeared in the 420,000 dalton fraction, also with an atomic ratio of 1:1. The 330,000 dalton molecules appeared to have a maximual binding capacity for the Cd-Se complex at a concentration of approximately 30 mol/ml of plasma while the 130,000 and 420,000 dalton molecules show a higher binding capacity. The appearance of the 330,000 dalton Cd-Se complex in these experiments is similar to the results obtained by Burk et al. after the administration of mercury as $HgCl_2$ and SeO_3^{2-}. As calculated by Burk, the atomic ratio of Hg to Se in these fractions was approximately 1:1.

Cadmium has been shown strongly to inhibit synaptic transmission, as do Hg and Pb, by depressing transmitter release from presynaptic nerve terminals. Evidence has also been obtained to show that synaptic effects of the heavy metals may be related to inhibition of the normal influx of Ca which triggers release of neurotransmitters.

A new study focuses on possible relationships between body burden of Cd in male and female rats and foetal development and subsequent behavioral characteristics in their offspring. So far no teratologic effects have been observed although some behavioral changes in the offspring have been observed. The Cd-fed parents showed some changes including elevated systolic blood pressure and dynamic pulmonary compliance in males. There was evidence also to emphysematous lung lesions. Additional lesions were found in endometrium and testes.

Administration of Cd, either orally as $CdCl_2$ or by inhalation as CdO, significantly reduces serum trypsin inhibitor activity and produces emphysema in male rats. In addition, CdO causes inflammatory changes in the lungs, which correlates with elevated lysozyme activity in pulmonary washings.

Efforts continue to estimate total body burdens of Cd with the aid of a tracer dilution technique. Evaluation of such body burdens remains one of the main problems in the study of Cd toxicology. The approach used on rats involves first prolonged exposure of rats to Cd followed by an equilibration period and finally by injection of a Cd isotope. The rate of isotope equilibration in various tissues is now being measured. If equilibrium can be reached, the specific activity of body Cd should provide a measure of total body burden.

Cadmium in Marine Organisms. Bearded seals and walrus from the Arctic ice pack are characterized by high cadmium contents and very low mercury levels in their tissues. The cadmium, mercury, and selenium contents of subcellular fractions from the two Arctic species were determined and the results compared with those obtained previously for the California sea

lion. Mercury showed a distinct tendency to accumulate in the particular fractions and in the high molecular weight proteins of the soluble fractions whereas cadmium occurred more in the soluble fractions, particularly concentrated in metallothionein.

The kidney and liver of bearded seal and walrus contained a mercury and cadmium binding protein of MW approximately 7,100 daltons, apparently identical to metallothionein. A specific assay to measure metallothionein levels in the tissues of three species of marine mammals, however, gave a very low degree of correlation between the metallothionein content of the kidney. These results are inconsistent with the inducibility of metallothionein by high mercury or cadmium concentrations in marine mammals.

Radionuclides. The plutonium content of human tissues was determined from a group of autopsy specimens from 50 individuals exposed to plutonium as a low level contiminant from nuclear weapons testing prior to the moratorium of 1963. These measurements are being made to assist evaluation of the significance of plutonium contamination that may be associated with future nuclear power programs. Samples of lung, liver, kidney and skeleton are available. To date, one set of tissues has been analyzed and plutonium was detected in the liver and vertebra. From the measurements it was estimated that 20 percent of the burden in this individual was in liver and the other 80 percent was contained in the skeleton. This result is consistent with observed animal experience and other reports of plutonium content in man. Total ^{239}Pu, ^{240}Pu content estimated for this individual is about 3 pCi.

An environmental radon instrument has been constructed which has been used indoors and outdoors in an area of normal radon content. It has been shown that radon in basements may be three to four times that found in the first and second floors and concentrations of 1 pCi/l and greater were observed. It is also likely that the concentration indoors on the second floor exceeds that outdoors by at least a factor of two. A diurnal variation of indoor radon has been shown in which the maximum occurs in the early morning hours. The doses to the lung from the natural levels of radon are the most substantial of any radiation doses found in the natural environment. This finding is, therefore, of great importance with respect to evaluation of the natural radiation background and its implications with respect to standard setting. It would appear that decisions regarding ventilation rates of structures should exert a large influence on the background dose to the lung.

Heavy radioelements are being measured in man both externally if the concentrations are sufficiently elevated or by radiochemical techniques if autopsy samples are available. The former technique has been applied to two cases of human accidental contamination with ^{241}Am, an adult and a 17 year old boy. The normal metabolism in man has been studied in these two people and the major loci of deposition of americium has been determined to be the skeleton and the liver. This was not unexpected based on studies in animals. The normal metabolism of americium has been modified in the two individuals by the administration of chelating agents,

particularly Ca-DTPA. The relative excretion in the adult male and the adolescent, presently undergoing rapid growth in puberty, has shown a marked contrast. Excretion from the male adult has been negligible whereas more than one-half of the skeletal burden of the adolescent has been eliminated. Studies were undertaken in baboons to see whether or not the excretion would differ in the juvenile and adult baboon and a similar pattern was found. It is hypothesized that the enhanced excretion in the juvenile results at least partly from the more rapid bone turnover rates in young people.

Metabolic studies have been continued in the baboon to elucidate the metabolism of heavy radioelements important in the nuclear fuel cycle. In particular, studies with ^{241}Am, in the baboon show that excretion is enhanced to a much greater degree in the juvenile than the adult baboon, especially with respect to the skeleton burden. However, in both juvenile and adult, the liver burden was excreted almost completely under a very short term period of chelation.

Studies with ^{241}Am citrate intravenously injected in the baboons showed that 35 percent of the injected dose is initially deposited in the liver. The efficacy of alcohol in enhancing the removal of ^{241}Am was studied in the baboon both alone and in combination with DTPA. DTPA produces a large increase in fecal ^{241}Am excretion attributable to a release from the liver. The effect of alcohol was to increase the excretion slightly but in a much less dramatic manner than DTPA alone or DTPA in conjunction with alcohol. It was concluded that alcohol is not particularly effective in enhancing excretion of americium from the liver.

Investigations have begun on the metabolism of curium, and alpha-emitting, bone-seeking actinide important in the nucleate fuel cycle. Curium-244 and ^{242}Cm are produced in amounts exceeding both americium and plutonium in nuclear power reactors, and accordingly it may be a more important radionuclide from the point of view of potential occupational and environmental exposure than had previously been believed. The metabolism of curium in the baboon tends to behave similarly to americium in the baboon based on six month measurements of two female baboons. At short times the retention time appears to be between 200 and 300 days in the skull, which is consistent with the observation of about one year for americium. The fractional deposition in liver appears to be somewhat less than that for americium.

Pyrethroids. Synthetic pyrethroids are now available with sufficient potency and photostability that they effectively control many important agricultural insect pests at dosages of 0.01 to 0.1 pounds per acre. They are the logical replacements for the chlorinated hydrocarbons that can no longer be used or are severely restricted in their use patterns. Prior to the registration and use of pyrethroids on a large scale, much more information is needed on their metabolism in mammals and plants, their bioaccumulation properties, and their photochemistry. The compound studied in greatest detail this past year is permethrin, 3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. Four labeled

preparations, two labeled in different positions in the alcohol moiety and two labeled in different positions in the acid moiety, were used to examine each of the trans- and cis-isomers of permethrin, i.e., eight labeled preparations were intercompared. Thirty distinct metabolites of trans, cis-permethrin were identified and synthesized, accounting for almost all of the products formed in rats administered permethrin orally. The major products of permethrin photodecomposition and of permethrin metabolism in plants and cows were also identified in studies that are still in progress. Investigations with houseflied and cabbage looper larvae have established unusual features of the insect metabolism, particularly in the principal sites of metabolic attack as compared with mammals. Studies have been initiated on the metabolism and photochemistry of the even more insecticidal α-cyano-3-phenoxybenzyl esters with pyrethroid-like activity. They appear to be characterized by slow metabolism and rapid photodecomposition relative to permethrin. Structure-biodegradability relationships have been compared for all important pyrethroids (44 chemicals) in the mouse liver microsomal esterase and oxidase enzyme systems, establishing pronounced effects of structural changes on the ease of metabolism. Current studies are emphasizing the stereochemical aspects of isomer and species differences in pyrethroid metabolism.

Toxaphene. Toxaphene has continued to be the focal point in the studies on chlorinated hydrocarbons since this insecticide is still the major chlorinated material used for insect control in the United States. Important advances were made in toxaphene analysis by the use of open tubular column gas liquid chromatography coupled with an electron capture detector that provides linear response over a wide concentration range and a computerized recording of retention time and relative peak areas. An examination of 22 samples of toxaphene and related chlorinated terpenes by this method establishes the high consistency of Hercules toxaphene manufactured from 1949 to 1975 and the large variations in composition of related materials from other manufacturers in the United States and abroad. An even more complete analysis of toxaphene components is provided by combining thin-layer chromatography with marker dyes and the open tubular column gas chromatography method. A method was devised to prepare gram amounts of one major toxaphene component, 2,2,5-endo,6-exo,8,9,10-heptachlorobornane, which provided material to use in studies on its alkaline degradation to two identified dehydrochlorination products and on its photodecomposition. The chlorination of camphene, tricyclene and various mono- and dichloro derivatives of camphene and bornane has provided ready access to other toxaphene components in studies that are in progress. It was also discovered on obtaining highly purified toxaphene components that the toxic properties are sometimes attributable to trace impurities rather than to major components.

In other studies, a toxaphene component (Fraction 2) has been separated into six sub-fractions by preparative glc. Last year it was reported that one of these sub-fractions was highly toxic and that another sub-fraction was equi-toxic with toxaphene to houseflies. The toxicities of three of the remaining four sub-fractions have since been determined. One sub-fraction is slightly more toxic, one is equi-toxic, and one is

significantly less toxic than toxaphene. Present efforts have been concentrated on the one highly toxic sub-fraction. (This sub-fraction is referred to as compound 2.) Though the structure of compound 2 is unknown, it has the empirical formula $C_{10}H_{10}Cl_8$. Based on these data it is speculated that compound 2 is probably the same as Casida's compound A (a mixture of 2 compounds). Compound 2 has been purified by preparative tlc followed by preparative glc. From this product a NMR spectrum has recently been taken and awaits interpretation. This should confirm whether or not compound 2 is the same as Casida's Compound A.

The toxicity to houseflies of toxaphene residues from crop extracts has also been studied. Zero day and 50 day extracts of cotton foliage have been tested. The LD_{50} of the zero day extract was 45 mg/kg as compared to 31 mg/kg for toxaphene. The LD_{50} of the 50 day extract was complicated by the presence of large amounts of green pigment. The extract was cleaned-up further and the LD_{50} determined as equi-toxic with toxaphene. However, in order to compare the toxicity of the two extracts the zero day extract will be cleaned-up in the same way as the 50-day extract. This is now in progress.

In vitro metabolism of pure toxaphene compounds using rat liver microsomes has been initiated. Using compound 2 the assay conditions have been determined and two metabolites tentatively obtained. These metabolites represent a de-chlorination and de-hydrochlorination of the parent compound 2.

In vivo metabolism of toxaphene and two tlc fractions corresponding to Fractions 2 and 7A have been studied in rats housed in glass metabolism cages. At this time all the results on the urine and feces have not been compiled and, therefore, no conclusions are available. From the tissue data it appears that very little activity remains in any tissue except for the two fat samples. In the case of the fat there is a difference in the storage between toxaphene and Fraction 7. Fraction 7 is stored at a higher level than both toxaphene and Fraction 2.

A study on cotton was initiated to determine the fate of toxaphene and its toxic fractions, along with total toxaphene, in a typical crop environment. Analysis also included DEF, a phosphorotrithioate defoliant and the plant desiccant paraquat. The most interesting results of this study were that during the first 50 days following application, toxaphene residues on cotton had over twice as much residue as the lint in the standing plant. This indicates some mechanical distribution of residue during the harvesting operation due to burr and foliage parts being imbedded in the lint; following the application of a desiccant the leaf portions dry up and concentrate the residues even further; analyses completed this far show very little if any decline of toxaphene and DEF in trash residues. Since DEF is a volatile compound and remained very stable in the trash, other pesticides which are generally less volatile would also be expected to remain.

Studies on toxaphene in soils have been under study for two years. There is definitely a different shift in the toxaphene profile as compared to air and plant studies. The mechanism appears to be a reductive process. In order to speed up this degradation profile, soils containing toxaphene were fortified with hematin and compared to those soils containing only toxaphene, following a two year degradation process. The results showed differences in comparison to the standard crude toxaphene, and had some similarity to the two year soil toxaphene residue.

Organotin Compounds. In the area of bioorganotin chemistry, the studies have now been completed on the metabolism of tetra-, tri-, and dibutyltin derivatives in the liver mono-oxygenase systems and in mice. Extensive destannylation occurs as the result of carbon hydroxylation at the α- and β-positions followed by destannylation leading to 1-butanol and 1-butene, respectively. A high level of biological activity is retained in the other products from hydroxylation of tributyltin derivatives, i.e., the γ-hydroxy, γ-keto, and δ-hydroxy compounds. The destannylation of tetrabutyltin is a metabolic activation process whereas the destannylation of tri- and dibutyltin derivatives involves detoxification. These general phenomena established with tributyltin derivatives were shown to be applicable to other primary- and secondary-aliphatic organotin compounds. Synthesis of (^{14}C) cyclohexyltriphenyl and a study of its metabolism in the monooxygenase system of rat liver established a perference for hydroxylation at the 4-position of the cyclohexyl group although some 2- and 3-hydroxylation also occurs. The synthesis of all of the possible hydroxycyclohexyltriphenyltin metabolites is nearing completion, so it should be possible to define the complete stereochemistry of the metabolic products. Preliminary metabolism studies have also been made on (^{14}C) tricyclohexyltin hydroxide (Plictran or cyhexatin miticide) and on (^{113}Sn) triphenyltin acetate (Brestan fungicide).

Dioxathions. The enzymatic and organismal basis for the rapid biodegradation of the trans- and cis-dioxathion isomers (important miticides) has been clarified in recently completed studies with rats and the photoproducts present on plants have been shown to be similar to those formed on peracid oxidation. In continuing investigations on bicyclic toxicants it was established that many 2,6,7-trioxabicyclo(2.2.2) octanes with P, P-O, P-S, C-H or C-alkyl substituents in the 1-position and C_2H_5, n-C_3H_7, n-C_4H_9, i-C_4H_9, sec-C_4H_9, t-C_4H_9 or other substituents in the 4-position act at very low dosages to produce convulsive seizures in mice. Other compounds that act in the same way to stimulate the central nervous system are: 2,6-dithia-1,3,5,7-tetrazatricyclo(3.3.1.$1^{3,7}$) decane-2,2,6,6-tetroxide; 1-(p-chlorophenyl)-2,8,9-trioxa-5-aza-1-silabicyclo(3.3.3)-undecane; bicuculine; picrotoxin; pentylenetetrazole. The findings of this study are relevant to the hazards associated with certain intermediates used in coordination chemistry and organic synthesis and with the combustion products of some flame retardants in the presence of certain polyols.

Phosphates and Carbamates. Progress continues to be made in defining the mechanism of teratogenesis induction in hen eggs by organophosphorus and methylcarbamate insecticides, particularly potent compounds being diazinon,

dicrotophos, monocrotophos, carbaryl and eserine. These compounds, sometimes at extremely low dosages, inhibit the synthesis of NAD from endogenous precursors. The metabolic block appears to occur between tryptophan and the pyridine derivatives. It can be bypassed by nicotinic acid or nicotinamide, effective alleviating agents for the teratogenesis. There is increasing evidence that this phenomenon is more important in avian embryology than in mammalian systems.

Male Swiss Webster mice were exposed to leptophos in the feed for 12 weeks at concentrations of 0, 10, 100, and 500 ppm. Signs of organophosphate poisoning were observed as moderate to severe inhibition of serum cholinesterase and decreased body weight gains of leptophos-exposed mice. Primary and secondary immune responses as measured by hemolytic plaque assay of antibody forming cells were not significantly altered. Histologically, pathologic lesions were not observed in mice which consumed as much as 170 mg leptophos/kg body weight/day for 12 weeks. It is concluded from this study that leptophos does not significantly affect antibody formation of mice and that this pesticide is of relatively low toxicity in this species.

In vitro binding of carbaryl of rat hepatic microsomes has been shown to occur in the presence of NADPH and O_2 and was inhibited by N_2, CO, reduced glutathione, cysteine, N-ethyl maleimide and SKF525-A. Binding was increased two- to three-fold by pretreatment of the animals with phenobarbital and 3-methylcholanthrene. Binding appeared to be dependent on the production of an active metabolite which was formed oxidatively in liver.

Studies on parathion are being conducted (a) to establish criteria for safe field reentry periods for crop workers with the organophosphate insecticides guthion, parathion, methylparathion and others; (b) to determine the fate of these pesticides on leaf surfaces; and (c) to determine their physiological effects, if any, on workers during normal work activities in treated fields.

In farmworker monitoring tests, no worker exhibited any sign or clinical symptom indicative of exposure to organophosphate pesticides. The daily group-mean RBC values for cholinesterase showed no statistical difference for values obtained over the seven week period. Findings were similar for group-mean plasma cholinesterase over the period of observation.

In the studies on residues on crops, preliminary results have thus far shown there were only moderate differences in the decay of those materials with the different applications. ULV applications resulted in higher dislodgeable residues on zero day, but after a few days the differences of dislodgeable residue levels were insignificant for both the parent materials and their respective oxygen analogs. The effect of dust on the leaves and the formation of oxygen analogs was of significant interest. The formation of both paraoxon and the oxygen analog of Superracide, GS 13005, did appear to be influenced by the dusting operation. In both cases the oxygen analog residues were higher with the dust by a factor of about 2x after seven days of paraoxon and 14 days for GS 13005.

Comparative Detoxication of Pesticides. Efforts were directed toward completion of two comparative detoxication studies of DDT metabolism by a marine and a freshwater crustacean. A second area of comparative research was begun with model compounds to elucidate specific biotransformation pathways as well as to attempt to categorize metabolic detoxication phylogenetically. A third objective entailed the investigation p,p'-DDE metabolism in a mammalian system (the rat) for later comparison with the invertebrates.

Aquatic studies were conducted in the continuous flow metabolism system reported previously. Nonpolar and polar metabolites excreted to the water flowing through the system were collected on non-ionic resin with approximately 100 percent recovery. Metabolites in solid phases were isolated by solvent extraction of homogenized fractions followed by lyophilization and reextraction constituents. For mammalian studies, all-glass metabolic chambers allowed separation of urine and feces which were then separated and characterized by tlc, glc, verified by mass spectrometry when possible and quantified by radiometry.

DDT was metabolized by both the marine isopod Limnoria tripunctata and the freshwater crayfish, Pacifasticus lenius culers. Dehydrochlorination to DDE, as well as reductive dechlorination to DDD, was observed in both species. In addition, polar metabolites chromatographing with DDA, DBH/DDOH, and DBP were evident, and fall into a logical detoxication sequence from DDD, paralleling metabolic pathways characteristic of higher forms, including man. These data, for the first time, provide knowledge of the metabolic fate of an environmental pollutant in unstressed, intact animals.

In the preliminary examination of model compounds, sea urchins (phylum echinodermata, an advanced invertebrate group) were exposed to p-toluidine (p-aminotoluene) as a relatively simple aromatic compound. In contrast to the known mammalian metabolism, the methyl group, the amino group, or the ring do not appear to be oxidized, while acylation of the amine and formation of conjugates accounted for 97 percent of the administered dose within 24 hours. The rapid metabolic rate was further supported by an uptake study which showed 7 µg of p-toluidine per gram of sea urchin were taken up each hour. The initial success with this model compound emphasizes the adaptability of low toxicity chemicals as metabolic probes, as well as the potential offered by lower aquatic forms as simplistic tools in human toxicology studies.

While DDT is not appreciably metabolized to DDE by either humans or the rat, DDE is most often the major body residue detected and apparently accumulated from food. Body residues are continually mobilized as a result of cell attrition or metabolic saturation. Some means of elimination must be active for this compound yet no breakdown pathway is known at present. This concept is being explored with particular emphasis on intermediate metabolites necessary for the elimination of DDE.

Examination of metabolites from rats dosed with (^{14}) p,p'-DDE indicated that approximately 10 percent of the administered dose was eliminated over a 10-day period. Urinary metabolites accounted for one percent of

this activity with two non-polar, and at least one polar, metabolites detected on tlc. Nine percent of the administered ^{14}C appeared in the feces. TLC separation of this fraction before and after acid hydrolysis indicated all metabolites were relatively polar with Rf values similar, but not identical, to those of DDA, DBP and DBH/DDOH. Major elimination of metabolites through feces emphasizes the importance of biliary excretion in this animal. In addition, the significant amount of polar metabolites suggests that DDE undergoes a variety of metabolic transformations although in limited amounts.

Dimilin. The increasing use of dimilin in pest insect control accentuates the need for defining its mode of action in blocking chitin deposition. Proposals that the block occurs due to alterations in metabolism of ecdysone and ecdysterone can now be discarded based on our recent studies. The block appears to occur in the steps between N-acetylglucosamine and the utilization of uridine-diphospho-N-acetylglucosamine in forming the chitin polymer. A simplified and rapid semi-*in vitro* system for studying chitin synthesis developed this past year offers promise of accelerating research on the mode of action of dimilin.

Herbicides. Important progress was made in defining the metabolic fate of two thiocarbamate herbicides, EPTC and Sutan, in rats by the use of (^{14}C) carbonyl preparations. The major metabolites of EPTC and Sutan are identical to those formed on administering their sulfoxide derivatives and the corresponding N,N-dialkylcarbamyl derivatives of glutathione and cysteine. Major metabolites include the carbamyl glutathione, carbamyl cysteine, carbamyl mercaptoacetic acid and carbamyl mercapturic acid derivatives. The persisting residues are unusually high in blood, suggesting carbamylation of a blood component. The carbamylated cysteine and glutathione derivatives are also formed in corn plants via the thiocarbamate sulfoxide intermediates. Thirty-two dichloroacetamides and related antidotes have been further examined in an extensive study comparing their activity as antidotes with their ability to increase the level of glutathione and activity of the glutathione S-transferase in corn roots. The findings of this study and of time and dose considerations for the antidote action further establish that the antidotes act by increasing the detoxification of the herbicides in corn plants. Studies on the mechanism of dichloroacetamide action are now underway in mammals.

Physical Factors

Light. Studies on the biological effects of environmental radiation were continued. The studies deal with several aspects of photobiology.

(a) Optical physics and instrumentation. Several scientist have been engaged in efforts to improve the efficiency, precision and specificity of instruments for photobiological studies for several years. During the past year they developed a meter

for determing the efficiency of light sources used in phototherapy and a portable sunburn ultraviolet meter (SUV). The meter for determining the efficiency of bilirubin destruction has proven to be a good predictor of light source efficiency in treatment of hyperbilirubinemia. The portable SUV meters have the same spectrum as units used previously but are much less expensive. Use of these instruments at stations located at seventeen sites throughout the world have shown a strong correlation between incident UV and thickness of the ozone layer. Whether or not an accurate numerical equivalent to ozone thickness can be derived is yet to be determined.

(b) Photochemistry. The spectral characteristics of several closely related photoproducts of aromatic hydrocarbon carcinogens are being studied. In addition, two fragance materials with demonstrated phototoxic activity are under investigation. Bergamot oil, the most widely known of the citrus oil photosensitizers, has long been known to contain appreciable quantities of xanthotoxin (5-methoxypsoralen), a potent photosensitizer. However, the studies indicate that phototoxicity of the oil is not confined to the xanthotoxin fraction. Rue oil, which does not appear to contain xanthotoxin or methoxsalen (8-methoxypsoralen), is also under study but the nature of phototoxic components has not yet been determined.

A recent report incriminated the widely used dye, ICI disperse blue, as a phototoxic substance via leaching from bathing suits and in industrial use. Tests showed that the material is not phototoxic by any route of administration when dissolved in water. However, in a standard test for photodynamic effect, it was found to produce photohemolysis.

Plastic "self destruct" materials have come on the market recently. Presumably a photoactive agent has been added to the polymer to accelerate photodegradation. Tests by irradiation with a solar simulator confirmed rapid degradation of the polymer. Extracts of the products with various solvents were not phototoxic when applied topically or parenterally. Photohemolytic tests are now in progress.

As part of the program in comparative photobiology, regeneration of newt limbs is being studied as a follow-up to the classic work of Professor Harold Blum. Regeneration is inhibited by irradiation with UV. Salicylic acid and indomethacin also inhibit regeneration, and the possible relationship to

prostaglandins is being explored. It is planned to use this system to evaluate the influence of photorepair and photosensitizers.

(c) Phototherapy. Contrary to reports of platelet inhibition in rabbits by phototherapy treatments, the phenomenon could not be produced in human newborns after 38-96 hours of treatment.

Studies of the effect of cycled and constant nursery lighting environment upon human growth hormone (HGH) were completed. A circadian rhythm of plasma HGH was found under both dark; light and dim; and bright light combinations, but this rhythm was destroyed under phototherapy and the concentration of plasma HGH was significantly reduced. No similar rhythmic variation was found in blood sugar or calcium under any of the three light conditions.

A prospective study was made of the influence of oxytocin induction of labor in humans. It was shown conclusively that the incidence and degree of severity of neonatal hyperbilirubinemia was more than doubled by oxytocin administration. This increased the need to administer phototherapy to the new born infants of such mothers to correct bilirubinemia.

Concern has been expressed that photolysis products of chlorofluorocarbons may produce significant depletion of stratospheric ozone. Model calculations, supported by some experimental data, indicate that atomic chlorine produced in the photolytic reaction catalyzes the breakdown of O_3 to O_2. The expected magnitude of O_3 depletion is controversial. The effects of Halocarbon 12 on the photolysis of high concentrations of O_3 in a dynamic system have been examined. O_3 was produced by arc discharge in a stream of O_2, at concentrations of up to 85^3 mg/l, and passed through a glass vessel with quartz end plates. When exposed to a xenon long-arc lamp a fraction of the ozone was destroyed. In one study, exposure at 15 cm from the source ($\sim$ solar constants) produced 8.5 percent loss in a stream containing 8.3 mg O_3/l flowing at 1.5 liters/min. Addition of halocarbon at 15 ml/min., which produced only the expected dilution in the absence of light, increased the loss on irradiation to 20 to 25 percent. Loss was directly proportional to halocarbon flow from 5 to 30 ml/min.

Addition of halocarbon downstream from the light source had no effect. This study confirms the possibility of photochemical interaction of halocarbons and ozone at significant rates, although it provides no direct evidence for the magnitude of such reactions under stratospheric conditions.

Microwaves. Studies on the biological effects of microwave radiation on the transport of dipolar molecules into the rabbit lens show no effect. However, irradiation is followed by an increase in extracellular water. These results support the hypothesis that the lens effects are due to microwave heating.

A system for irradiating cultured cells and investigating membrane transport during irradiation in a temperature-controlled environment has been fabricated and calibrated. The same system that controls temperature is also used to measure absorbed power. A mathematical model for the temperature distribution within the irradiated sample has been developed. The plot of the log (efflux of 3-o-methylglucose) vs. temperature for rabbit erythrocytes shows a change in slope near 22^{o}.

RESEARCH HIGHLIGHTS

Regular Research Grants Program

Presentation of the highlights of the Regular Research Grants Programs follows the format of the previous year's report.

Of particular interest is the initiation during the past year of a new research program, the Young Environmental Scientist Health Research Grant Program. This program was designed to encourage young investigators to develop an interest in problems of environmental health sciences and to provide support for their initial independent research efforts.

The initial receipt date for applications of March 1, 1977, was announced in the January 1977 issue of the NIH Guide for Grants & Contracts.

The goal of the program supporting research in the areas of mutagenesis and carcinogenesis is to safeguard the genetic heritage, reproductive capacity and well-being of progeny of man against environmental toxicants. Studies include identification of substances in the environment which may adversely effect the genetic make-up of man, development of reliable test systems and research to elucidate molecular and cellular mechanisms in mutagenesis and reproductive anomalies.

Detection of Mutations

Drosophila. One of the important systems for detecting genetic mutations has employed the use of *Drosophila*. Using this species a large number of diverse genetic tests have been developed. A number of tester stocks have been developed to provide a series of genetic tests from the same treated individual, thereby, permitting a comparison of genetic response for one test as, for example, sex linked recessive lethal versus translocations. This type of work has limited value until one of the genetic tests can be related directly to the dose. One study has used the sex linked recessive lethal test in conjunction with dosimetry employing ethylmethanesulfonate (EMS). The advantages are that the dose response curve can be used to distinguish among theories for mode of action of a mutagen and is particularly important in extrapolating the genetic effects to a low-dose level. Secondly, the proper determination of dose should provide a basis for comparison among different species, thereby permitting the comparison of the effect of model mutagens among different species. Third, after accumulation of a data base of dose versus response in a number of different test systems using several model mutagens, it will be possible to make a quantitative prediction as to the genetic consequence of a given level of alkylation in the germ line of man. It has been demonstrated that a dose square relationship can be excluded for the range of dose of 8×10^4 to 5×10^6 ethylations per sperm cell, achieved by an exposure to a feeding concentration ranging from 0.1 mM to 25 mM. Since a dose squared relationship can be excluded, so also can multiple alkylations be excluded as a normal requirement for the mutation induction, and instead, adhere to the hypothesis of an alkylation of a rare site as the mutagenic event.

A study is underway to determine whether or not *Drosophila* is capable of identifying suspect chemicals that might pose a genetic threat to man and whether *Drosophila* will be a reliable and realistic model for assessing their effects. To date, *Drosophila* is the only *in vivo* animal system which enables the efficient testing of both transmitted gene mutations and chromosome aberrations in germ cells. *Drosophila* has been used to systematically study comparative mutagenesis with 15 representatives of various categories of directly and indirectly acting mutagens and the detection of potent mutagens is largely dependent upon the genetic end point employed as a measure of mutagenicity. It is felt that this observation is of significance when evaluating testing procedures. Using a well defined tester strain of *Drosophila* (Berlin K), 27 compounds have

been tested for mutagenicity during the past 12 months and it has been demonstrated that rapid testing of potential mutagens is feasible in Drosophila.

Studies designed to select new mutant strains of Drosophila melanogaster which are potentially defective in mechanisms of DNA repair, have recently demonstrated a high correlation between the mutagenicity of an agent and its carcinogenic potential, suggesting the direct involvement of DNA repair mechanisms in preventing the development of a cancerous state. It is felt that the isolation and characterization of mutagen-sensitive strains should provide not only valuable organisms for screening environmental agents for potential mutagenic or carcinogenic activity, but also provide considerable insight into the mechanisms of higher eucaryotes employed for repair of damaged DNA. Nearly 20,000 EMS mutagenized X chromosomes have been examined for new mutations which confer increased sensitivity to killing during the developmental life cycle by the alkylating mutagen methymethansulfate. To date approximately 50 new mutants have been isolated and these new strains have been grouped into five different complementation groups.

A variety of biological systems for testing compounds for mutagenic potential are being investigated. Included for testing for mutagenic effects are such compounds as saccharin, caffeine, the inhalant anesthetic halothane and the drug metronidazole which is used as an antitrichomonal and is one of the 200 most widely used prescription drugs in this country. Preliminary studies have led these investigators to surmise that Drosophila could perhaps serve as a demonstration of a novel technique for monitoring the environment for the presence of mutagenic substances.

Eukaryotic Organisms. Another study is designed to establish the procedures to be used in detecting chromosomal aberrations in an eukaryotic microorganisms which would be suitable for large scale screening of drugs and chemicals. The study employs a tester strain of Aspergillus nidulans to determine the conditions which would allow for detection during both mitosis and meiosis: (a) recessive mutations, (b) non disjunction, (c) crossing over, and (d) translocations. Studies to date using the mitotic system as a screening device has shown that ethanol causes chromosomal aberrations in the organism employed. At a high level of resolution specific types of damage have also been recognized. These findings are significant in light of the epidemiological correlations between esophageal carcinoma and ethanol consumption. Studies on hydrochlorothiazide, in addition, suggest an epidemiological search for clinical correlation of malignancies with the use of this diuretic.

Human Lymploid Cell Cultures. A modified host-mediated assay system using cultured human lymphoid cells as target cells for screening chemical mutagens or carcinogens has been developed. Induction of a chromosome damage of the target cells will be used as indicator of possible mutagenicity or carcinogenicity of a given compound. The investigators are using cells from six human cell lines studied for their growth potential in a diffusion chamber after implanation in

the peritoneal cavity of mice. The rate of sister chromatid exchanges (SEC) is being used as an extremely sensitive index for measuring DNA damage by certain chemicals. The rate of sister chromatid exchange in human cells cultured in diffusion chambers implanted into mice, which were then injected with cyclophosphamide (CY) were studied. The results indicated that the rate of SEC is correlated positively with the dose of CY. The SEC in human cells *in vivo* described by this technique may provide a valuable system for testing the activity of chemicals on chromosomes, especially those which need to be metabolically activated.

Mammalian Lymphoid Cell Culture. The development of a mammalian lymphoid culture system for mutagenicity testing is also being explored. Chemicals to be tested will be monitored using a mutation system involving *S. typhimurium* to develop an activation mixture which is non toxic to mammalian cells and to begin to isolate immunoglobulin variations of the marine myeloma cell variants induced by chemical mutagens. The *S. typhimurium* assay system has demonstrated mutagenesis at several doses of an acridine mustard and of an alkylating agent ethylmethane sulfonate, both of which have been subsequently used to produce mutation in mouse myeloma cells. Post-mitochondrial fractions from human lymphocyte and mouse myeloma cell lines have already been prepared and are now being tested for their capacity to activate the procarcinogen 2-florenamine. The significance of these studies is that both the human lymphoid and the murine myeloma systems showed increasing promise of providing a reliable means of testing for mutagenicity at specific loci in mammalian cells.

Chinese Hamster Ovary Cells. Another testing technique involves the use of Chinese hamster ovary cells to study mutagenesis *in vitro* and to demonstrate how such systems can be useful in predicting the nature of putative genetic alterations imposed by environmental agents on the human community. This system differs from most mutagenesis studies using mammalian cells in two fundamental respects. Instead of stating the genetic effects of agents at a single locus, this study involves variants for over 50 gene products within a single cell and, moreover, the system can distinguish between genetic changes which modify the gene product (structural gene mutations) and those which alter the expression of a locus (control or regulational events). It has been shown that enzyme low side products resolvable on starch gels are under regulational control in cultured cells and this control may be modified by exposure of cells to mutagens. These investigators have also isolated a total of four different mutant lines which they feel indicates the power of the system not only in testing mutagens but in developing marker cell lines. Moreover, multiple banded patterns consistent with the subunit structure of the enzymes have been isolated for six different enzyme loci products, demonstrating the functional diploidy of most genetic loci in this long term cell line. The appropriateness of such aneuploid cell lines for genetic studies is, therefore indicated, and the suggestion by some that the high frequency of mutation in these cell lines using selective systems is due to functional haploidy is made less likely.

Autoradiographic Techniques. Further development of rapid autoradiographic measurement *in vitro* of DNA repair synthesis in human cells is under

investigation, to make it a more efficient and reliable means of predicting and evaluating the genetic effects of hazardous agents in humans. A study has been conducted with leukocytes from 20 normal human donors to measure background levels of unscheduled DNA synthesis (UDS) and the extent of UDS after *in vitro* exposure of the cells to 10^{-5} molar 4-nitroquinoline-N-oxide. These levels of UDS were measured after the samples were processed by the rapid autoradiograph technique. It was determined that the polymorphonuclear leukocytes are a more sensitive cell type than either stimulated or unstimulated lymphocytes because of the greater nuclear area of the polymorphonuclear cells. These findings tentatively indicate the suitability of measuring UDS in the polymorphonuclear cells to screen human population groups, since variations in standard values for background rates or response to the 4-nitroquinoline could be detected. Specifically, an increase in background rates indicate exposure to a hazardous agent and a decrease in response to the compound might indicate either a defective repair mechanism or exposure to an inhibitor of DNA repair. These studies are continuing.

Umbilical Cord Cells and Blood Cells. A study is underway to compare the rates of chromosome breakage and rearrangement (CBR) in cells from umbilical cord and peripheral blood of low-birth weight and control infants and to investigate alternate methods of assessment of chromosome damage, specifically rates of SEC, and to correlate them with the observed rates of chromosome breakage and rearrangement. In addition, an attempt will be made to correlate high rates in breakage, or other evidence for chromosomal damage, with maternal or infant exposure to potential environmental mutagens. To date, there has been an investigation of the relationship of culture time to evaluation of chromosome breakage and rearrangement using the sister chromatid exchange technique. It has been revealed that contrary to expectations a culture time of 48 hours is too long for the study. It was found that a significant fraction of lymphocytes cultured from umbilical cord blood are in the second division after 48 hours and for this reason the culture time of 40 hours is preferable. The study assumes, that if an increase frequency of damage is detected in umbilical cord blood, it may reflect effects of environmental factors acting during pregnancy. If damage is detected in postnatal peripheral blood, it may reflect effects of diagnostic or therapeutic regime used for premature or growth retarded, low-birth weight infants. The investigation will also serve to evaluate a new method for detection of genetic damage in populations.

Mechanisms of Cancer Induction

Role of Metabolites. A great number of substances found in the environment are being studied in relation to cancer induction. The mechanism of cancer induction by vinyl chloride, trichloroethylene, and related compounds are included in this group of compounds. Emphasis is on the formation of chemical and biochemical reactions of the metabolites of these compounds as the evidence accumulates which indicates that the metabolites are the activated carcinogenic intermediates rather than the parent halo alkenes. An unexpected finding is the fact that two potential metabolites of 1-chloropropene reacted with quanine at the 2-amino and 3-nitrogen

groups. Many alkylating agents react to the 2-amino and 1-nitrogen, but this is the first case found of alkylating agents reacting up to 2-amino and 3-nitrogen. This finding may be significant in the carcinogenesis of vinyl halides. An additional finding is that a metabolite of trichloroethylene, most probably an epoxide, alkylated liver protein and DNA, and there is a clear correlation between such binding and tumor induction caused by trichloroethylene. The importance of metabolism of foreign substances in human cancer induction is emphasized by the trichloroethylene studies in rat and mouse liver.

Polychlorinated Biphenyls (PCB). The polychlorinated biphenyls (PCB) have caused a worldwide ecological problem. There have been incidences of high level exposure of man to these compounds producing widespread ill effects and persisting for an indefinite period. Recent studies in nonhuman primates have revealed serious effects on reproduction in adults, and altered learning in behavior in infants exposed to low levels of PCBs. Moreover, hepatocellular carcinomas are complications of chronic PCB exposure in rats. Since man is continually being exposed to the PCB compounds, it is important that a more thorough understanding be obtained of how these compounds effect the body, and the mechanism by which these effects arise. An understanding of the absorption, tissue distribution and excretion of two common PCB isomers in nonhuman primates and rats has been gained. In addition, the metabolic fate of these compounds has been partially clarified. One of the isolated metabolites of transdihydrodiol, suggests the metabolism of at least a portion of the PCBs, are through an electrophilic arene oxide intermediate. These findings suggest a possible mechanism for the binding to cellular macromolecules and for the toxicological and carcinogenic action of the PCBs. It has been shown that the PCB metabolites do in fact bind to the cellular macromolecules and produce transformation in cell cultures. These studies are providing a basis for understanding the possible mechanism of PCB action on mammalian tissues.

Aflatoxin. Studies designed to provide an understanding of the metabolism of aflatoxin B_1 (AFB_1) including both the activation and deactivation processes and characterization of the enzymes involved in the metabolism of AFB_1 and the study of the mechanisms of their actions are being carried out. Studies on the effects of species, strain, age, nutritional status, hormonal, physiological and environmental manipulations on the metabolism of AFB_1 are expected to provide explanation at the molecular level for the reported differences in aflatoxin toxicity in carcinogenesis due to these factors. The data produced by these studies will be of value in setting up tolerance schedules for aflatoxin and related toxic substances. Moreover, the studies on aflatoxin metabolism and the relationship to mutagenesis and carcinogenesis should define more precisely the role of metabolism, metabolic activation *in vivo*, and micromolecular binding on DNA damage, for aflatoxins and related chemicals. During the past year it has been shown that there is a good correlation between *in vitro* metabolic activation (DNA binding) and mutagenesis and carcinogenesis. An *in vitro* DNA binding assay has been modified and scaled down making it possible to carry out 10 to 15 assays a day. For chemicals that can be tritiated or detected in small amounts by spectral

absorption or fluorescence emission, in vitro DNA binding assay provides a quick means for screening some of the drugs and environmental chemicals for their carcinogenic and possibly mutagenic potential. Studies in mice have shown that the genetic regulation of aflatoxin B_1 metabolism to AFM_1 will provide new leads to the understanding of the mechanism of action of AFB_1 and development of model systems with predicted value in mutagenesis, carcinogenesis, and toxic evaluation of aflatoxin and related compounds. Moreover, these studies should provide a basis for developing methodology to study genetics of AFB_1 metabolism in easily available human tissues such as lymphocytes and monocytes.

Physical Factors

The effects of the various physical factors on human health are largely unknown, however, there is an increasing awareness of these effects as the result of recent studies on such factors as noise, heat, and cold microwaves, laser radiation and light.

Noise. One such study focuses on the relationship between physical characteristics of noise and the resulting noise induced (temporary) hearing loss. Determination of some of these relationships will allow the specification of octave-band levels of noise spaced across the audible range of frequencies that will not produce a hearing loss regardless of the duration or number of exposures and the validity and range of application of the equivalent power model of noise-induced hearing loss. The study involves exposure of human subjects, under quasi normal conditions, to diffuse sound fields of varying intensities, and measurement of the noise induced hearing loss and its psychophysical and physiological correlates. It was felt that by determining the quantitative facets of noise induced hearing loss, the establishment of noise standards and criteria becomes much simpler than in the past. In addition, the measurement procedures have a scientific basis. Data suggests that the contour which depicts levels of noise that will not create a risk of a temporary hearing loss is flat or nearly so from 500 hertz to 4.0 kilohertz. The critical levels are at about 75 S.P.L. Moreover, the weighting network to measure sound levels should, therefore, also be flat from 500 hertz to 4.0 kilohertz. Psychophysical tuning curves, that is, the masking of a pure tone by pure tone shows that when the frequency of the masker was lower than the frequency of the signal, the 24 hour noise exposure had no effect. The masking data, therefore, question the validity of the traditional measurement of noise induced hearing loss; namely, auditory thesholds for pure tones.

Noise/Drug Interactions. Another study on the effects of noise, centers on the possible synergistic effects of combinations of noise and noise/drug, since the prevailing damage risk criteria (DRC) treats each type of noise separately even though they rarely occur separately in real life. The purpose of the investigation is to determine the limits of the interaction of continuous and impulse noise; for example, what

levels, durations and spectra of the two noises are necessary for the interaction to occur? What are the possible mechanisms for the interactions and how do noise/interactions relate to noise/drug interactions? Since salicylate, in the form of aspirin is by far the most common drug ingested today and produces a temporary loss of hearing and other symptoms associated with cochlear losses, animals were exposed to combinations of sodium salicylate and either high level short duration noise or low level long duration noise or impulse noise. It was found that under all conditions the combination groups manifested no interaction and the hearing loss was simply a reflection of the dominate ototraumatic agent. The results are important because they show that salicylate ingestion does not present any additional hazard to the auditory system exposed to noise. This group of investigators is also studying early changes in the cell membrane of the cochlea, since this is the first cellular component to be touched by noise. It is felt that studies of the membrane properties (e.g., membrane binding characteristics, permeability, and etc.) will add to the fundamental knowledge of the cochlear sensory cells.

Cold Adaptation. The mechanisms involved in adjustment and acclimatization to cold and heat and their effects on human health are being studied from several aspects. One project has as its objective the study of the sympatho-adrenal responses occurring during seasonal acclimatization to cold and to compare these with responses occurring during acclimation to cold in the laboratory. Specific seasonal adjustments in sympatho-adrenal activity, assessment of the importance of these adjustments for the maintenance of homeothermy and elucidation of possible functional capacities and changes which may be unique adaptations in artic-subartic mammals in which large adjustments to seasonal thermal requirements must be made, are included. These investigators have studied the calorigenic response to injected norepinephrine compared as an index of non shivering thermogenesis in groups of voles which were summer, fall, and winter acclimatized and tested metabolically at thermoneutrality (25° C) and during cold exposure (5° C). It was found that the winter acclimatized voles showed a significantly greater metabolic response to norepinephrine than summer voles at both 25° C and 5° C. Thus, thermogenesis during cold exposure, and resulting from exogenous norepinephrine, appears to be based on the same mechanism, and norepinephrine has thermoregulatory significance in these animals. Other studies have shown that after subcutaneous injection of L-norepinephrine-H3, both cold acclimated voles and cold exposed summer voles had increased rates of decline of norepinephrine-H3 in all tissues. The results of these studies should provide a greater understanding of the role of the sympathetic nervous system and adrenal medulla in the development and maintenance of enhanced non shivering thermogenesis and the maintenance of homothermia in animals. Adjustments to cold and other seasonal factors may frequently be a problem to humans who must travel or establish residence in cold climates, and adjustments must be made to seasonal extremes, as in the subartic or artic in Alaska. The research should provide further insight to mechanisms involved in successful adjustment to cold.

Physiological Response to Heat. The direct effects of heat on physiological responses in women are being investigated as the relationship

of aerobic power (VO_2 max) to womens physiological responses to work in hot environments. It was found that the single factor which best discriminated between the trained and untrained woman was the ability of the athlete to maintain her stroke volume, and thus her cardiac output, well beyond the time at which the untrained females had to be removed from the environmental chamber. These studies have suggested several areas for further investigation into the mechanism by which cardiovascular fitness training enables some women to meet the challenge of acute exposure to work in the heat. For example, one of these areas would be to look at the patterns of fluid shifts during work in the heat for both trained and untrained women.

Laser Radiation. The increase in the use of laser technology has shown a need for additional studies on health effects. One such study is concerned with the elucidation of photochemical and photophysical aspects of the biological effects of laser radiation and, more specifically, with utilizing the laser as an investigative tool in photobiology. The health hazards associated with laser radiation, as well as its potentially significant therapeutic applications, clearly establish the need to understand the consequences of laser excitation at the molecular level. It is felt that laser techniques can provide new insights into the details of photochemical and photobiological processes and apparatus is being developed for applying these techniques in systematic studies of biologically significant molecules.

Microwave Irradiation. Pre- and postnatal effects of microwave irradiation are being studied by measuring modulated and pulse transmission of frequencies to determine the effective dose and monitoring procedures to be used. Exposure levels are being determined during the implantation period, the organogenic period, and the fetal period that do, and do not, result in embryopathy. Emphasis has been on designing and developing the necessary equipment, the anechoic chamber and appropriate monitoring systems. These have been developed and base line studies both of the teratologic and behavioral nature have been carried out on control animals. The importance of this investigation rests on the fact that exposure of the general population to governmental, industrial, and commercial sources of chronic low levels of microwave irradiation has been increasing significantly over the past decades. The present investigation is focusing on the chronic low level microwave irradiation upon pregnant females to determine whether the embryo can be utilized as a sensitive indicator of the non-thermal effects of this form of radiation.

Radionuclides. The increase emphasis on energy production in this country and accompanying health hazards has spurred investigation on the effects that radionuclides in the environment from power production will have upon a local population and upon the global population. The critical nuclides for nuclear power are the alpha emitters and the beta emitting fission product crypton-85. Studies are underway to provide internal dose estimates for exposure to these emitters, via air-food pathways. The dose estimates are derived for a particular cell type since it is known that certain types of tumors are most significant. For the alpha emitters the dose estimates will be to basal cells in bronchial epithelium in the upper

bronchial airways and to the osteoprogenitor cells on bone surfaces. For the beta emitter, crypton-85 which is soluble in fat within bone marrow, the critical cells are the hemopoietic cells as well as the osteoprogenitor on bone surfaces. In order to determine an accurate dose to the hemopoietic cells in the bone marrow and the osteoprogenitor cells on bone surfaces, the energy loss function for crypton-85 beta particles in a tissue like medium have been established. The dose from KR-85 is small compared with that from naturally occurring RN 222. The health effects from these exposures for the world population can be determined using the estimated absolute risk of leukemia presented by the Beir committee. At equilibrium, assuming a constant annual dose, the committee estimates 50 cases of leukemia per 10^6 person REN. The number of cases of leukemia resulting from 7.5 mrad per year or 75 mrem per year, alpha dose, RN 222 in a world population of 3.6 x 109 persons, would then be 13,500 cases.

Photosensitivity. An investigation is underway to recognize and assess environmental agents which cause adverse cutaneous photosensitivity reactions in man, to develop new biochemical and biophysical tests for the recognition and assessment of photosensitivity diseases, to study the mechanism of action of these adverse photosensitivity reactions in laboratory animals and the steps to take to prevent and/or ameliorate adverse photosensitivity reactions by means of systemic and/or topical photo-protective compounds. This group of investigators participated during the past year with the FDA and the National Program for Dermatology in selecting and publishing data concerning those agents which were suspect and for which appropriate photo-patch test could be instituted. This work resulted in the compilation and publication of an appropriate manual, "The Role of Patch Testing in Allergic Contact Dermatitis." Studies have also indicated that Dowicide 33 is a potential photosensitizer. This agent is currently used extensively by janitors and gardeners. The program for developing new biochemical assays for the recognition and assessment of protoporphyrin abnormalities (lead poisoning EPP, iron deficiency anemia) is now operative on the clinical level. This research continues to furnish material to the FDA and to the National Programs for Dermatology concerning techniques for examining and ascertaining the magnitude of photosensitivity reactions.

Odorant Irritants. Investigation is continuing to explain the mechanisms and significance of perceived intensities of odors and irritants. The overall objective is to characterize how human beings perceive the intensity of odorant irritants. Various experiments explore the olfactory and the common chemical (trigeminal) attributes of sensations produced by N-butyl alcohol. These two attributes combine in an almost linear fashion to produce the overall perceived intensity of the stimulus. It was found that the common chemical intensity made only a small contribution to overall magnitude at low concentrations, but its proportional contribution increased with concentration. Similarly, a speed of response to the common chemical attribute increases more rapidly than that to odor. Nevertheless, odor always made its appearance sooner than the common chemical attribute. Repeated inhalations caused the odor intensity to decrease but caused common chemical intensity to increase. The data obtained from normal subjects

studied agreed with those obtained previously from subjects with unilateral destruction of the trigeminal nerve. Also the psychophysical data agreed with the neurophysiological data on the activity of the olfactory and the trigeminal nerves; and finally, the ease with which subjects could judge each attribute independently of the other made it possible to compare directly how the two different chemosensory systems respond to exactly the same input. The significance of these experiments is that they seek to provide a quantitative basis for odor evaluation and control, particularly in relation to increasing the effectiveness of warning agents and decreasing the effectiveness of noxious vapors.

Metals

Cadmium. Cadmium is a byproduct of zinc and lead production. It is used in electroplating, pigments, alloys and pesticides. Industrial effluents and motor vehicle exhausts contribute to the atmospheric burden of this compound. Cadmium is also a water pollutant as a result of industrial release and leaching from galvanized copper or plastic pipes. Its use continues to climb as does the refining of zinc and lead. Hence the potential for environmental exposure to this material continues to increase, particularly in industrial areas.

Metallothionine. The metabolism and biochemical actions of cadmium are being determined in feeding studies. The effects of dietary factors and the induction of biological defense mechanisms, especially the generation of cadmium binding protein (metallothionine) is under investigation. It has been found that low cadmium intake increases cadmium uptake, emphasizing the importance of adequate calcium in the diet.

The effects of cadmium on carbohydrate metabolism are being studied in part, by investigations on the effects of this pollutant on pancreatic function. It has been determined that metallothionine (cadmium binding protein) appears rapidly in the pancreas after a single dose of the metal is administered. By administering cadmium as a pretreatment, it was shown that, although cadmium exposure reduces the glucose induced secretion of insulin from pancreatic islets, the prior induction of metallothionine serves as a protective role against subsequent exposures. Glucose tolerance of mice was not affected by chronic cadmium treatment.

Tissue Distribution. The toxicity and distribution of sublethal oral doses of cadmium chloride have been determined. At two days, tissue concentration was highest in liver followed by intestine, kidney, pancreas, spleen, heart, lung, testes, muscle, brain, blood and plasma. After two weeks, all tissue concentrations decreased 50 percent except for liver (which remained unchanged) and kidney (which increased threefold). Metallothionine concentration in the liver remained constant from days 2 to 14 but increased over the same period in the kidney. Testicular injury was noted. Fecal elimination was the most important route of excretion, controlled by biliary flow.

Bone. If lead intake exceeds the body's capacity to excrete the material, the excess tends to deposit in bone tissue. The factors which govern the movement of lead into and out of bone is under investigation, as the results may lead to methodologies to increase the excretion of the metal from poisoned individuals. It has been determined that the hormones which regulate bone mineral metabolism also have an effect on lead deposition and removal. In bone cell culture, hormones (parathyroid hormone-PTH or calcitonin-CT), or Ca^{++}, PO_4^{++}, or Mg^{++}, all affected the release of deposited lead. The results are supportive of clinical observations of dietary effects on lead turnover from bone.

Metal Binding Proteins. The induction of metal-binding proteins and their localization in subcellular components following exposure to cadmium is being correlated with the intracellular movement of cadmium. The effects of cadmium and other metals on subcellular function (e.g., DNA polymerization) is being studied concurrently. This investigation has shown that the uptake and disappearance of labeled cadmium in nuclei is in concert with the appearance of cytoplasmic cadmium binding protein. Thus it appears that cadmium is the effector for this protein.

Cytotoxic Effects. Inhalation studies of cadmium are being carried out to study mechanisms of uptake, alveolar cell distribution and alterations in macrophage function. Since the lung is an important portal of entry for airborne toxic metals, these studies of the lung's primary defense mechanisms will yield new knowledge about the pathogenicity of this material. To date, techniques for the treatment of alveolar macrophages have been completed and rate of uptake studies are in progress. The effects on uptake biochemical parameters such as oxidative phosphorylation and glutathione concentration have been measured. The cytotoxic effect (as measured by macrophage viability) of cadmium has been determined.

Lead

Storage batteries, fuel additives and pigment manufacture are the major uses for lead which expose human populations to this toxic material. Neurological, renal and hematological toxicities are manifestations of chronic or sub-acute lead exposure.

Renal Pathology. Mechanisms of renal damage due to lead ingestion is being studied by several grantees. Using renal clearance measurements, it has been determined that a significant fraction of lead in plasma will be filtered by the kidney. However, much of this filtered lead is reabsorbed by the kidney tubules. Induction of metabolic alkalosis markedly increases lead excretion, suggesting reabsorption by combining with an organic anion of a weak acid. Since the kidneys are the major route for excretion of lead, such information may lead to better methods for protecting against lead accumulation and for facilitating lead excretion.

The effects of low level lead ingestion on renal function in dogs were studied by measuring renal blood flow, filtration rate and sodium excretion. No differences between control and exposed animals were observed. This observation was confirmed in rats. However, the sensitivity of the lead-exposed animals to antidiuretic hormone (ADH), was decreased. This was determined by observing a decreased ability to concentrate urine when water intake was restricted in lead-exposed rats. Therefore, although low level lead-exposure does not alter the early maturation of glomeruli or renal circulation, alteration in water and electrolyte balance may occur prior to overt manifestations of toxicity such as aminoaciduria.

Uroporphyrinogen. The ability of a material in liver cytosol to protect uroporphyrinogen synthetase (an enzyme important in heme biosynthesis) against inhibition by lead chloride has been observed. Isolation and characterization of this factor has been completed (it is a polyglutamated folate derivitive). Although this factor failed to prevent the lead-mediated inhibition of other enzymes in the heme pathway, the factor appeared to protect against uroporphyrinogen synthetase inhibition by other metal ions such as cadmium, zinc and mercury.

Heme Biosynthesis. It has been determined that acute lead administration inhibits enzymes responsible for liver microsomal heme formation and stimulates the enzyme which breaks down heme, hence, the associated reduction in cytochrome P-450 activity. Chronic administration of lead had no such effect on microsomal heme, but did cause the well known inhibition of erythrocytic heme biosynthesis. This indicates some sort of hepatic adaptive effect which is under study.

Marked variation of heme pathway enzymes with increasing age has been observed. Young animals and animals with high rates of red blood cell production were shown to have high levels of ALAD, an enzyme considered indicative of lead poisoning. Likewise, the age of red blood cells affects the concentration of erythrocytic enzymes.

Metabolic Indicators. A project is underway to evaluate the clinical significance of metabolic indicators of lead poisoning, investigation of clinical significance of slightly elevated blood levels, evaluation of possible metabolic effects detectible in individuals with normal blood lead levels and the evaluation of the mechanism of interference of lead with heme synthesis. A test has been developed called the Free Erythrocyte Porphyrin (FEP test) for the detection of childhood lead poisoning and of lead poisoning in general. The FEP as a screening test is presently being used on a nationwide basis to detect childhood lead poisoning. There is some indications from the most recent observations that even at blood lead levels considered "normal" and observed in the majority of the urban American Population, there is evidence of metabolic effects of lead. The FEP measurements have also provided useful basic information on the physiological significance of heme metabolism defects in such a rare syndrome as erythropoietic protoporphyria as well as in the extremely common lead deficiency of childhood.

X-Ray Quantitation. A technique for quantification of lead levels in bone is being sought. A computer assisted X-ray imaging technique is under study using human subjects from a community lead poisoning prevention program. Readings are correlated with clinical measurements such as blood lead levels.

Behavioral Studies. A broad spectrum of behavioral characteristics of primates is being surveyed after exposing animals for the first year of life to elevated but subclinical levels of lead to determine whether any long-term behavioral changes or deficits are produced. These effects will be related to the known body burdens of lead and any clinical medical changes noted in the primates. Studies to date have indicated that there is a difference in the behavioral characteristics of animals which have been exposed to different levels of lead. Three groups of animals were used at five weeks of life compared with a controlled group of animals. The high lead and low lead level monkeys had twice the locomotor activity of the controls at one year of age. This normalized in all but two of the three high lead monkeys by two years of life. The high lead monkeys were socially submissive and showed more fear type behaviors in mild stress tests. In addition, the high lead monkeys learned a two choice object discrimination problem at the same rate as controls, but were retarded in learning a first reversal on a problem, although not generally retarded on learning subsequent reversals on a problem. On a test of visual acuity under different light levels, the high lead monkeys have proven to be essentially blind when dependent exclusively on rod vision (scotopic visibility). This is similar to results seen in adult monkeys treated with methylmercury and appears to be an enduring deficit. It is in line with reports of blindness from acute lead poisoning. Tests are also being carried out on endocrine function in lead treated monkeys and it has been found that there is an abnormal response to insulin challenge in which the high lead monkeys failed to recover blood glucose levels as rapidly as controls and show no rebound of blood glucose to supernormal levels as with controls.

Neurotoxicity. Neurological changes due to lead exposures are commonly observed toxicities. Lead encephalopathy is the first neurotoxic observation in acute cases. Studies on the flow of lead across neural barriers are in progress. Calcium flow measurements have been made to serve as controls. It is postulated that this is a parameter affected by lead. By tyrosine accumulation studies it has been determined that the choroid plexus accumulation is affected by lead to a much greater extent than the brain. Hence, "selective barrier dysfunction" may contribute to the pathogenesis of lead encephalopathy. By being able to clinically determine these dysfunctions using sensitive biochemical indicators on cerebrospinal fluid, low level neurotoxicities may become detectable.

The effect of drugs on overt lead encepalopathy is being studied in conjunction with psychological tests and morphological observations of lead-poisoned animals. EDTA and BAL treatment improved lead-poisoned animals clinically and morphologically. Decadron had no significant effect and vitamin D enhanced bone absorption of lead. Visual discrimination tests and motoreactivity measurements are being correlated with these data.

Congenital lead intoxication by maternal dosing of lead is being studied to determine gross and microscopic pathologies in the developing nervous system. Localization of lead in the fetal nervous system and effects of dosing at various times during gestation is being determined. Electron microscopic techniques have been developed which have identified the deposition of lead to be localized in the cyto-membrane systems (nuclear membrane, mitochondria and endoplasmic reticulum). This may represent the mechanism for toxicities more subtle than the consequences of vascular damage such as cerebral hemorrhage, edema, and degeneration. A description of analysis of the effects of lead and mercury ions on synaptic transmission in the isolated frog sciatica nerve--sartorius muscle preparation is being attempted. The approach is designed to produce the kind of data which ultimately will be required for localizing the site of action of lead and mercury and for adequately understanding their neurotoxic effects. It is felt that experiments to date have given firm evidence that lead competitively antagonizes the action of calcium at the nerve terminal. This finding is further substantiated by the observation that the blocking effect of lead on the end plate potential is reversible. The significance of these studies are that lead and mercury which have been used in the experiments to alter processes underlying both pre- and postsynaptic events in neuromuscular transmission should eventually provide a relationship to a particular physiochemical parameter of a series of divalent cations to a given measure of synaptic function. In this way, it may be possible to localize the action of a particular chemical moiety within the pre- or postsynaptic membrane and relate to a synaptic function. The effects observed in the experiments could account for some of the neuromuscular disorders of chronic lead and mercury poisoning.

Lead Chelation. A program to design and synthesize therapeutic agents for the treatment of lead and mercury poisoning has shown some promising results. Thiolithocholic acid and thiodeoxylithocholic acid (mercapto steroids) quickly removed mercury and lead from *in vitro* solutions. Mice injected with methylmercury and treated with BAL had a 43 percent survival *vs* 0 percent for controls. The survival rate was 67 percent when methylmercury treatment was followed by thiocholesterol. Lead treated mice likewise benefited from treatment with thiocholesterol and/or thiolithocholic acid. Metabolic and excretion studies on these compounds are now underway.

Other studies underway to develop chelating agents designed to be more effective than those in current use in combating the injurious effects of metal poisoning involves the determination of the crystal structure of a number of complexes formed by toxic metal ions and potential chelating agents, with emphasis on the ligands BAL, cysteine, penicillamine, and their derivatives. In penicillamine chemistry, investigators have developed a new synthetic technique for examining the coordination of penicillamine to metal ions in the environment similar to that found in the biological situation. In the living system, the toxic metal ions bind to the peptide residues of the proteins, and so complexes which contain metal ions coordinated to both aminoacid and β-penicillamine are probably better models for the biological activity of this ligand than are complexes of the free metal ion. The synthesis and crystal structures of the first fully characterized complexes of this general type have been achieved.

The penicillamine ligands in the complexes which have been studied are all dianions, deprotonation occurring at the carboxyl and sulfhydryl groups; the same sites of deprotonation have been observed in the diionic penicillamine and cysteine complexes of the other metals. These apparently are the first examples of complexes in which Pen^{2-}--acts as a tridentate ligand to a metal ion. It is felt that the stereochemical selectivity observed in these reactions may be of far reaching biological significance. The studies strongly suggest that D-penicillamine is a much more effective chelating agent than L-penicillamine. Hence, the study provides a second compelling reason for choosing D-penicillamine over DL-penicillamine in therapy and should also serve to warn clinical scientists that test results obtained with DL-penicillamine may not apply to D-penicillamine.

Mercury

Neurotoxicity. The mechanism of mercury encephalopathy is under investigation. Early studies indicate that organic mercurials depress protein synthesis in cerebellar synaptosomes isolated from rats during late stages of mercury induced encephalopathy. Aminoacid uptake and incorporation (an index of protein synthesis rate) showed that various aminoacid rates are affected differently by methylmercury. Furthermore, alterating potassium and sodium ion concentrations changed the uptake and incorporation rates, demonstrating the independence of their processes.

The margin of safety between our present body burden of mercury and the level when fetal exposure produces morphologic, neurophysiological, and behavioral defects is being studied. The principal embryopathy effect of chronic low dose exposure of the developing fetus to methylmercury is a decrease in size and weight. Studies have also shown that the congenital exposure to mercury does more than diminish the intrauterine nutrition of the fetus. Fundamental cellular mechanisms are apparently permanently damaged. It would appear from these studies also that the primary lesion in the nervous system related to methylmercury exposure is the degeneration of neuronal cytoplasm. The objectives of one study are to compare and contrast the effects of exposure to low-doses of methylmercury upon developmental characteristics and central nervous system function in two species, the domestic chicken and the rat. It is hoped that this study will give a better understanding of the interactions of methylmercury compounds and central nervous system development and function to provide a better understanding of tragic human exposures which have been encountered in Japan, the United States, and Iraq. The data could help to provide information so that the appropriate government agencies can set the maximum allowable limits for these compounds and to establish the reliability of the use of lower forms of life in determining the potential of other substances for inducing behavioral or functional teratogenic consequences in humans. Administration of methylmercury to the one day pregnant rat resulted in a dose related appearance of the compound in the uterus at five days of pregnancy. From the data, it was predicted that 55.5 percent and 26.6 percent of the dose given on the first day of pregnancy would result in equivalent concentrations in the

fetal or perinatal brains. At no time do these doses cause decreased viability of the developing rat embryos, fetuses, or neonates. Studies in the chick embryo indicated that a critical period for functional deficits produced by methylmercury may exist in the chick and it is during the first trimester of development that they are most sensitive. In addition, initial studies started with rats born to mothers administered methylmercury on day 0, 7, or 14 of pregnancy indicate a possible critical period during which liver function may be permanently impaired.

Mechanisms. The mechanisms of organomercurials is also being studied in mammalian cell culture. The growth rates, mitotic indicies and subcellular components of methylmercury treated cells are under investigation to establish the "target" of the organomercurial at the molecular level. The molecular parameters of chromotin (interphase chromosomes) are also being determined as a baseline for measurements subsequent to mercury treatment.

Chelation. New antidotes for mercury poisoning are being designed and evaluated. Low molecular weight and polymeric chelating agents are being synthesized and compared with BAL by LD_{50} measurements in mice. Two polymers have been found which are superior to BAL and N-acetyl-DL-penicillamine in its ability to raise the observed methylmercury LD_{50}. These compounds are themselves of low toxicity.

Selenium

Studies on the mechanism of selenium accumulation in plants are attempting to differentiate the abilities of these varieties of plants to incorporate selenium into their proteins. The role of cysteinyl and methyionyl-tRNA synthetase is thought to be the key to this process. It was thought that accumulator species had less ability to incorporate Se into proteins and by storing selenium in non-protein aminoacids would avoid the inhibition of vital protein catalyzed processes. It has been found that this theory may be an over-simplification. Selenium tolerance appears to have more than one mechanism, since selenocysteine was not available as a protein source to a selenium accumulator plant. Studies of this tolerance are continuing.

A study is underway to determine whether selenium plays a role in the hepatic microsomal cytochrome P-450 system, and if it does, what that role is. These investigators reported last year that selenium is involved in the induction of this system by phenobarbital. More recent work has indicated that the impaired induction of P-450 is associated with marked induction of hepatic microsomal heme oxygenase indicating increase breakdown of heme in phenobarbital treated selenium deficient liver. An attempt has been made to identify selenium containing proteins in the liver microsomes.

Arsenic

Arsine (AsH_3) gas use in industry has greatly increased in recent years. This compound is known to be hemotoxic, and the mechanism of hemolysis is under investigation. It has been determined that erythrocytes exposed to arsine show a dose dependent increase in osmotic fragility, even after exposure is terminated. A direct decrease in membrane acetylcholinesterase activity was also observed. These observations will now be correlated with data from individuals working at an arsine plant to see if parameters for exposure screening can be developed.

Arsenic compounds are being isolated and characterized following formation by algae grown in a medium containing inorganic arsenic compounds. It is well known that trace elements, among them arsenic, are taken up by living matter, incorporated and chemically changed. Such transformations can produce compounds even more toxic than the derivative to which the organism has been exposed, or can serve as a detoxification mechanism. Very little, well founded, information about such transformations is available. Lower life forms such as algae which occupy a position at the bottom of the food chain can, therefore, protect higher organisms from the detrimental effects of trace elements, or, on the other hand, pass on a compound more toxic than one absorbed from the inorganic environment. It is expected that the knowledge gained through these studies will contribute to the elucidation of the true toxicity of arsenic and its compounds and perhaps help to decide whether or not arsenic is an essential trace element. A wide variety of algae have been tested in order to select the one species which is most suited for mass culturing in the presence of sodium arsenate or arsenite, develop the appropriate growth medium, determine the highest arsenic concentration in which the selected algae can be successfully grown, and to develop the proper conditions for mass culturing the selected species under controlled conditions. Pilot studies have indicated that the organism Tetraselmis chuii was the most appropriate one for these studies. Conditions for their growth and culture and extraction of arsenic compounds have been worked out and will be applied in mass culture.

Vanadium

Studies on vanadium in basic solution are designed to provide an understanding of the role of arsenates and vanadates in biological systems. The work on vanadium continues to provide insight into the electronic and molecular structure of vanadium compounds and provides the basis for continuing work on vanadium in neutral and basic solutions. The results obtained included the first evidence relating to the mechanisms of hydrolysis or alcoholysis of arsenate or vanadate esters and the first determination of the structure of an arsenate ester.

Molybdenum

An investigation is being made to understand and structurally characterize molybdenum complexes which have possible relevance to understanding the coordination chemistry of molybdenum in enzymes. Particular emphasis has been given to determining the structures of binuclear molybdenum complexes of ligands with sulfur donor atoms. The most significant results obtained from this study so far has been the discovery of binuclear molybdenum complexes containing three bridging atoms. The discovery of triply-bridged MO (V) complexes means that such species can now be realistically proposed as possible entities in the reactions of molybdo-enzymes.

Metal Synergism

The potentiation of mercury, cadmium and lead in combination is being studied by measurements of lethal dose, chromosome damage and histological effects on kidney, liver and spleen. Initial experiments have shown cadmium to be additive to the toxicity of mercury, lead to be synergistic with the toxicities of mercury and cadmium, and mercury and cadmium to be protective of subsequent mercury and cadmium exposure. These data are interpreted to demonstrate that given the affinity of these metals for SH groups, pretreatment with a less toxic metal appears to protect from the toxicity of the more toxic material. Initial triplet experiments support this hypothesis, and the work could be of significance in developing preventative measures in working environments.

Metabolic Studies

Investigators are attempting to obtain a better understanding of the processes involved in heavy metal metabolism, particularly focusing on the unusual protein metallothionein, and its involvement in the *in vivo* metabolism of metabolically essential and toxic heavy metals. These investigators are developing a radioimmunoassay for rat cadmium thionein, zinc thionein, and copper chelatin which will be used to determine the potential ubiquity of these proteins in other tissues besides liver and kidney and to provide a better understanding of possible transport of the proteins in the plasma. There has been success in producing antibodies to the three rat liver proteins in rabbits, using the precipitating gamma globulins against zinc thionein, cadmium thionein, and copper chelatin. The radioimmunoassay is being developed for the proteins.

Ozone. Several NIEHS grantees are working towards a better understanding of ozone toxicology. Mechanisms of action studies to determine the primary point of oxidant attack are being carried out on cellular plasma membranes. The hypothesis is, that deleterious effects are due to damage to the membrane causing a loss of ions, particularly potassium. Methods to mimic this type of damage and to measure membrane integrity have been developed. By understanding the mechanisms of tissue damage and repair, methods to ameliorate ozone hazards may become evident.

Another study using human red blood cells demonstrated the possibility of crosslinking of membranes following ozone-induced lipid peroxidation. The measurement of this effect as it relates to ozone and ozone plus NO_2 dose is being determined. Results will be useful in determining ozone concentrations which may be considered "safe" or "hazardous."

NIEHS-supported research has also shown that red blood cells lyse rapidly when exposed to ozonized phospholipids but not when exposed directly to ozone. Enzymic effects have also been determined. This indicates the possibility that ozone also penetrates the cell and reacts with intracellular protein. Although the red blood cell is simply a model of pulmonary cells which are primarily affected, the similar structure of membranes and cellular proteins makes this model appropriate.

The effects of combinations of air pollutants are important to understand since the usual human exposures involve breathing a multiplicity of pollutants in an urban environment. Studies on guinea pigs (an experimental animal known for its susceptible respiratory tract) has indicated that sulfur dioxide is not synergistic with ozone despite reports that ozone assists the conversion of the SO_2 to sulfuric acid. Similarly, oil mists did not appear to intensify the effects of breathing 1 to 10 ppm SO_2. However, various salts of trace metals, particularly copper salts, appear to be potent potentiators of SO_2. The effects of common air pollutants on lungs at concentrations comparable to those existing in urban areas of many of the present day larger cities are being studied. The experimental design offers the considerable advantage of investigating the effects of environmental stress on a compromise organ that consist of tissue in the state of increased stress (i.e., compensatory emphysema) and of increased susceptability to damage. In this respect the findings may have particular significance in the area of neonatal development and in the case of pneumonectomy in the adult. It was found that the increase in tissue mass and volume of the lungs as a result of normal growth is balanced by progressive increases in the number of alveoli in rabbits. This probably levels off at body weights greater than 9.0 killogram. While pneumonectomy in the rabbit results in an increase in the size of the alveoli of the remaining lung, exposure of a normal lung to ozone (at least in rats) causes a reduction in alveolar size due to a decrease in lung compliance. A combination of pneumonectomy and exposure to ozone results in a "sniffer" lung with a reduced lung volume. The timing of the onset of exposure to ozone following pneumonectomy will be critical in terms of the intensity of the response.

SO_2. The mechanism of sulfur dioxide's adverse effect on lung tissue is being characterized. It is believed that biological exposure to SO_2 may initiate the oxidation of important coenzyme systems which supply the driving force for important biochemical processes. The molecular species responsible for the oxidation of NADH and NADPH has been identified. Another grantee is studying the reaction of SO_2 with biological disulfide bonds. Rates of this reaction in various tissues and the biochemistry of these processes are being determined. These studies continue to elucidate the reactions caused by SO_2 exposure and should be useful in determining how tissues cope with such stresses.

The effects of exposure to common atmospheric pollutant agents (SO_2, NO_2, formaldehyde and mixtures of these) upon alveolar macrophages by biophysical and biochemical techniques are being studied and characterized. Included are in vivo exposure of rats and in vitro experiments with alveolar macrophages obtained from a subhuman primate, the baboon. Interesting results have been obtained on the effects of inhaled SO_2 upon alveolar macrophages and the lung surfactant systems. The results of initial exposure to SO_2 appeared to indicate a more consistent effect of length of recovery period than duration of exposure. The macrophages and surfactant from five animals which were terminated immediately upon removal from the exposure atmosphere were obtained and the rate of phagocytosis was measured. The cells were still capable of phagocytosis after exposure, although the initial rate of phagocytic uptake was lower than prior values for Fisher rats. The apparent depression of phagocytosis did not appear to be associated with any significant alteration in cell viability as measured by dye exclusion. The most pronounced variation of any parameter examined was in macrophage ATPase activity which was elevated at the end of the exposure, decreased to approximately control levels within three hours, rose to a peak between 24 and 40 hours, fell again, but remained slightly higher than controls at the end of 142 hours post-exposure. In experiments in which rats were exposed to a tenfold lower concentration of SO_2, an elevation of macrophage ATPase activity was again observed as was marked increase in intracellular lysozyme levels. Studies are continuing on alveolar macrophages which have been isolated from nucleotides from baboons. The cells are being characterized by biochemical and physiological parameters. It is expected that these studies will contribute information regarding the alveolar macrophages and their many roles in the maintenance of health of the individual, in face of exposure to air borne pollutants.

SO_2 and Particulates. An extensive epidemiological study is being carried out with data being collected from selected sites to determine the effects of particulates and sulfur oxides in combination, on adults and children. During the past year, two communities, St. Louis, Missouri, and Steubenville, Ohio, were surveyed. Final selection sites for surveys have been made for the last two communities in this study and they are Topeka, Kansas, and Portage, Wisconsin. Preliminary data have been processed from the first three sites and a paper on the methodology and preliminary results is in preparation. Indoor/outdoor monitoring was done for mass respirable particulates at the Watertown site last summer and the results indicate that the values at the central station, indoor and outdoor at persons homes and personal dosimeters carried by individuals gave similar

values. A similar study in the winter showed that the indoor values were different from the others, higher if the family smoked and lower if they did not.

NO_2. Nitrogen dioxide, another major urban air pollutant, is likewise under investigation. The effects of NO_2 on lung tissue is being studied by determining the rate of RNA synthesis and turnover in alveolar macrophages. Results indicate that RNA synthesis rates are most affected. This indicates the possibility that RNA polymeron may be the primary lesion point in NO_2 inhalation, and this knowledge may lead to the discovery of protective agents.

NO_2 and Particulates. The mechanisms of experimental emphysema is under investigation by the analysis of exposure of the lung to NO_2 with and without fly ash in both the hamster and the cat. In addition, the effects of a systematically administered carcinogen, diethylnitrosamine (DEN) is being studied, to evaluate the susceptibility of the injured and regenerating tracheobronchial and alveolar epithelium to agents which produce neoplasia. Morphometric studies have shown that there is an increase in total pulmonary resistance and up stream resistance which amounted to approximately 140 percent of controls in the NO_2 cats and 120 percent of controls in the NO_2/dust animals but unchanged in the animals receiving dust alone. Exposure of animals to diethylnitrosamine suggest that exposure of NO_2 and dust, in addition to DEN, may stimulate additional tumors in the trachea and not in the larynx. There are preliminary indications that there are no major differences in distribution on tumors in the tracheobronchial tree, lung and liver in the animals receiving DEN/NO_2/dust as compared to those receiving DEN only.

Vitamin A. Epidemiological as well as recent experimental evidence suggests that the development of lung cancer is the result of complex interaction of a number of exogenous and endogenous factors. Besides the carcinogenic insult of environmental factors modifying the target organs, nutritional factors such as vitamin A and host immune state may be of paramount importance in initiating the disease process. It is felt that nutrition may have an important role in air pollution on the health effects of pollutant gases. Vitamin A deficient animals are more susceptible to nitrogen dioxide gas exposure. The deficient animals when exposed intermittently showed marked lymphopenia and at the same time activated certain latent bioagents. This effect was more severe in animals exposed to NO_2. Non exposed hamsters with vitamin A deficiency did not exhibit this mark of lymphocytolic effect and hepatic degeneration. Vitamin A might play an important role in activating viral genome and at the same time may play a possible synergistic association occurring between these two environmental and host factors. The occurrence of the adenomatous growth in NO_2 exposed and vitamin A deficient hamster emphasizes this finding. It is felt that further research is urgently needed to elucidate this important observation since it may have a similar implication to that found in human cancer epidemiology, in that, both NO and NO_2 can form nitrous acid which then combines with amine to form nitrosamines. The nitrosamines are the major suspect in urban cancer known to man. The statistical correlation found between nitrogen dioxide and high incidences of cancer in many urban areas

underscores the importance of this finding. Recent studies indicate that a much higher level of vitamin A may be needed by urban dwellers working in densely polluted areas. The depletion of vitamin A among the exposed hamsters was much faster than in non exposed animals. This finding suggests the need to critically reanalyze the essential vitamin A and other nutritional requirements for urban population.

Vitamin E. The possible protective effects of vitamin E or lipid ingestion from the pulmonary toxicities of ozone or nitrogen dioxide are being documented. The hypothesis is that these materials may ameliorate the oxidative effects of air pollutants on unsaturated lipids in the lungs. Dietary regimens to raise the "peroxidizability index" or to increase the vitamin E content of the lung did not clearly demonstrate alternation of ozone toxicity. However, certain distal effects of ozone inhalation were observed. Hepatic cytochrome P-450 levels appeared to be reduced, as indicated by phenobarbital sleeping time. Increased utilization of arachidonic acid occurs post ozone exposure (or vitamin E deficiency), which may have some secondary hematological effects.

CO. Carbon monoxide effect on the oxygen-carrying capacity of blood is well-known. However, other effects have received less study. One such effect is the potential for CO to combine with other heme containing molecules, such as cytochrome P-450. It has been determined that rats exposed to 250 ppm CO adapt by not only increasing hemoglobin content, but also by increasing liver cytochrome P-450 content. In addition, postnatal development of hepatic mixed function oxidase in rats has been found to be retarded by exposure to carbon monoxide.

Another study involving carbon monoxide has as its objective the investigation of the biochemical pathways involved in the metabolism of dihalomethanes. These compounds are widely used in commerce and industry for a variety of purposes and have been considered to be of relatively low order of toxicity, however, studies have shown that the dihalomethanes are metabolized to carbon monoxide, a compound that is well-known to be highly toxic. During the course of the studies on the metabolism of dihalomethanes to carbon monoxide, it was observed that the halo forms are also metabolized to carbon monoxide. Present studies suggest that the halo forms are metabolized to carbon monoxide by a hepatic microsomal cytochrome P-450 mixed function oxidase system. Using isolated rat hepatocytes, the reaction was increased in cells isolated from phenobarbital treated rats and was inhibited by SKF 525-A. These studies suggest that the isolated rat hepatocytes may be a good model for future covalent binding studies. Studies on the metabolism of haloforms are of public health significance because these compounds have recently been identified as contaminants of municipal finished drinking water supplies.

Further studies on the sensory and behavioral deficits in animals exposed to low concentrations of carbon monoxide using operant conditioning and psychophysical techniques are being carried out. The studies emphasizes the relationship between the physiological and behavioral mechanisms which occur under exposure to CO. The changes which are noted in the laboratory

animals may be extrapolated to infer, that under such conditions, there may be an interference with human work efficiency, judgment and general motor skills.

Photochemical Oxidants. Work on photochemical oxidants is proceeding. The qualitative and quantitative irritant potential of chemical mixtures associated with photochemical smog are being determined. Human and animal sensitivity to u.v. irradiated hydrocarbon/nitrogen dioxide mixtures generated from propylene, butadiene, 1-butene, cis-2-butene and ethylene were determined. Concentration-response relationships for acrolein and formaldehyde were also developed (they are the two main photooxidants produced in photochemical smog), and it was found that these compounds are antagonists, possibly reacting at the same receptor site. Animal models of hyperreactive individuals to sensory initants have also been developed for further study.

The acute health effects of sulfate, nitrate and hydrocarbon aerosols, prevalent in the urban ambient environment, are not well defined. Experimental methodology for the generation, characterization and use of these aerosols and exposures of monkeys to these pollutants has been developed. Using these dosing techniques, dose-response relationships will be obtainable.

Particulates. Many investigations are being carried out to elucidate the relationships between aerosols containing particulate matter and various respiratory diseases. These studies include a consideration of particle size deposition and chemical composition of the particles. One such study is attempting to determine how particulate matter which reaches the alveolar surface effects the potential lung defense functions of alveolar macrophages. This is essentially a three part study involving in vitro cultures of alveolar macrophages to isolate the activity of these cells from other lung defense mechanisms to examine the process by which macrophages incorporate particles, and finally, to study the means by which inert particles might block or partially block alveolar macrophages and the consequences of such a block in both immunological specific and non-specific areas. Using rabbit alveolar macrophages exposed to fumed silica with a mean mass diameter of 0.007 microns for one hour, the cells appeared to be coated with silica as observed by scanning electron microscopy. The generally recognized mechanism of silica toxicity in macrophages is that phagocytosis of the silica particles is followed by a silica induced lysis of the resulting phagolysosome. This lysis finally leads to lysis of the macrophage and recirculation of the particle. The studies indicate that the extremely small particles of silica are not phagocytized but, instead, coat the plasma membrane.

The role of the pulmonary alveolar macrophage is seen as the primary cellular defendant in the lung, carrying out phagocytosis and intracellular digestion. Accordingly, investigative efforts have focused on both the qualitative and quantitative aspects of these processes. It has been shown that after alveolar macrophages have ingested inhaled particles (as well as some particles reaching the lungs after ingestion or after injection), most of these cells are cleared from the lung by way of the bronchial tree. The

greater part of the uncleared material in the lungs finds its way by lymphatic and other routes into pulmonary connective tissue macrophages located primarily in the investments of the larger airways. It is believed that in retaining undigestible biologically active (radioactive, carcinogenic) particles, the intracellular digestive system of the connective tissue macrophages remains chronically activated, and by concentrating the material, the cells foster a catabolic environment locally. The findings help to explain why some types of emphysema are centriolobular in origin, and why in many species the main pulmonary carcinoma is bronchogenic. As alveolar macrophages are clearing through the bronchi, many of them contrive to pursue their customary scavenging activities on the bronchial surface, while others die and release their contents, some of which appear to pass through the bronchial epithelium, eventually to reach the pulmonary connective tissue macrophages. These cells, therefore, receive particulate matter by more than one pathway.

A predictive theory has been developed for the aerosol deposition in the human nose during inhalation. The primary mechanism of deposition is due to particle inertia. The deposition probability at each region is calculated for particle size ranging from 0.1 millimicron to 10 millimicron and for a flow rate between 10 liters per minute to 30 liters per minute. It has been shown that the principal sites of deposition are the nasal hairs and the nasal valve. More recently, revisions have been made in the theory of aerosol deposition in the human lung. Quantitative calculations based upon this theory were carried out for both steady state breathing and single breath washout. The results are that there is good agreement between theory and experiment and the details have been published. The theory of aerosol deposition in the lung largely depend upon the mixing between the tidal air and the residual air. To understand this mixing process these investigators have undertaken a theoretical study of air mixing in a model lung alveolus. It was found that the mixing occurs only when the alveolar shape varies during breathing and when the inspiration time is different from the expiration time. Under normal breathing a 5 to 10 percent air mixing is expected. Using the same model a theoretical study has been conducted on aerosol deposition in the lung during breath holding. Since diffusion and settling are the only deposition mechanisms involved, it has been found that the aerosol persistence in the airways during breath holding is to be calculated with the use of a combined deposition efficiency. It has been shown that the upipolar charge carried by an aerosol particle has a significant effect on lung deposition. An analysis was made for deposition due to steady breathing of diffusional particles carrying an unipolar charge. It was found that for 0.33 millimicron unit density particles with 29 electronic charges per particle, deposition increases by 42 percent during steady breathing. The mechanism for this increase is the presence of the image force between the particle and the airway surface.

A standardized mammalian test system for assessing hazards associated with inhalation of respirable dust and air pollutants in man is being developed. An esophageal collection technique is being used for collecting all material (particle cells, mucus and so forth) cleared from the pulmonary system of the rat to the oropharynx. The collections technique is accomplished by intubating the esophagus through the stomach. No surgery on

any portion of the pulmonary system is involved. Studies to date have shown that a single particle exposure results in a low percentage of dust laden cells in the lungs. Less than 10 percent of the pulmonary cells recovered by lung irrigation contained particles. During the current year studies were conducted to evaluate the effect of repeated particle exposures on this process. The aims were to increase the number of dust laden cells collected each hour and to stimulate the pulmonary cell population so that the subsequent response to a single particle exposure made after stimulation would result in increase cell clearance. The results of the repeated particle exposure experiment showed that this technique resulted in an increase in percentage of dust laden cells in the sample. In addition, a detailed study on the mixed salivary transfer of iodide and pertechnetate ions following a single intravenous injection have been completed. It was surmised that these ions which are known to be transferred in the saliva, would serve as positive controls for subsequent work. In addition, the effect of treatment with perchlorate, which alters the distribution of these ions in the body was tested. The results confirm and extend available data on the clearance on these materials via the mixed saliva and emphasize the importance of this pathway in the translocation of solubilized material in the body. The evaluation of the hazards associated with inhalation of toxic particulate matter requires knowledge of the lung clearance mechanisms and the fate of material remaining in and leaving the lung, and of factors capable of effecting these processes.

A time of flight aerosol beam spectrometer capable of measuring the composition of various size sub-ranges of the atmospheric aerosol is being developed. A vacuum optics chamber has been designed and built. This chamber is, therefore, the heart of the measuring system. It is expected that this instrument will be able to detect and measure the size and composition of particles in the range between about 0.2 or 0.3 millimicron and about 10 millimicron diameter or more. This size range contains most of the material that penetrates into the working regions of the lung. It is expected that the instrument will be capable of identifying certain materials present in the atmosphere which are toxic or possibly toxic, from a public health point of view.

Studies are continuing to determine the distribution pattern of particles according to breathing patterns and changes in regional deposition resulting from these breathing patterns. This process is being addressed by using a servo-mechanism respirator to impose specific respiratory patterns on excised, preserved dog lungs. It has been shown that lungs can be preserved in an arsenic-borax solution and that they maintain the flexible properties of the tissue over a long period of time. The lungs will be ventilated with a servo-mechanism and presented a radioactive aerosol at the trachea. The distribution of radioactivity is then measured using a gamma camera. Finally, a modified vibrating orifice aerosol generator has been constructed which produces a monodispersed aerosol from any material that can be put into solution form. It is now being used as a primary standard to calibrate the aerosol centrifuge and as one of the aerosol generators in these experiments.

The retention of methylene blue aerosols by positive casts of the rat nose has shown that the two major loci of impaction, in addition to the area just inside the vestibule are the posterior dorsal region of the nasal cavity and an area near the entry of the suprapharyngeal tube. It is clear from these studies that nasal retentions previously calculated and based on simple assumptions regarding air flows in nasal cavities are in serious error. Lung volume measurements have been completed for mice, rats, guinea pigs, and rabbits. These measurements are in satisfactory agreement with the volumes calculated from measurements of the casts. Using these results, one may determine the times when diffusion deposition and settling are needed rather than the combined deposition probability described by Landahl which is applicable when one of the deposition processes is highly predominant. This study is of importance for the study of the behavior of small (less than .5 micron) dense particles in the small airways. These studies will provide a rationale basis for interpreting the toxicity of aerosols in laboratory animals in terms which will enhance the reliability of predictions of the hazards for man.

Patients with lung cancer apparently exhibit a larger quantity of asbestos bodies in the lung than a control population of healthy individuals. Specimens from 56 patients with lung cancer were analyzed, of these, 13 have shown counts greater than a hundred bodies per gram. Counts have been performed for 80 control patients but interviews with patients or families to obtain detailed smoking and occupational histories are still in progress. Of the lungs from 29 patients with gastrointestinal cancer, eight have more than a hundred bodies per gram. An attempt has been made to make a correlation of light and electron microscopic counts of asbestos fibers and it was found that there was no correlation between these two parameters. The purpose of these studies is to define the extend of the contribution of low levels of asbestos exposure to neoplasia. It was felt that by correlating asbestos body counts with occupations not currently recognized as dangerous, it may be possible to define populations at risk, let alone cancer.

An attempt is being made to discover specific molecular effects of certain oxidizing agents likely to exist in polluted air (including singlet molecular oxygen) on lipid components (such as cholesterol) of biological membranes. Insight could be gained into the nature of molecular injuries to membranes and to cells, thereby suggesting means of offsetting such deleterious effects of polluted air. A study in the oxidation of cholesterol by the one and two electron reduction products of molecular oxygen; namely, superoxide radical anion and peroxide anion have been completed. These studies were undertaken in order to determine whether either species would specifically oxidize cholesterol under conditions stimulating biological conditions. There was no evidence that the superoxide radical anion readily attacks cholesterol. Since no oxidation products of superoxide attack on cholesterol were noted in the alkyl systems. It is concluded that superoxide radical anion is not an important source of singlet biological oxygen, a matter which is currently subject to much speculation in the published literature.

Epidemiological Studies. Attempts are being made to correlate visits to emergency rooms for respiratory disorders and air pollution data in New York City. To date, studies have shown that there is a poor correlation found between pairs of stations in New York City and emergency room visits for respiratory causes and air pollution in the two inner city areas. These negative findings are thought to be of significance because it has demonstrated that no one station in New York City can be taken to represent the daily fluctuations of air pollution throughout the city and it seems conclusive that the results of studies based upon this assumption are imprecise and most likely invalid. Correlation coefficients between daily averages for both sulfur dioxide and smoke shade for all pairs of stations of the aerometric network were calculated by month or season, and are low. These low correlations could not be explained by taking into account heights of stations or geographical proximity between stations. The aerometric stations, therefore, do not adequately describe the day-to-day variation in the levels of air pollution of the areas they were designed to represent.

Pesticides/Herbicides

The influence of carbamate herbicides on microtubules and membranes in animal cells is under study. In the first year, a fluorometric technique was established which can detect microtubule-membrane interactions. It was determined that microtubule function and assembly in leukocytes in vivo is prevented by certain compounds. These techniques are continuing to be utilized in studies of toxic materials.

The pesticides and PCB's are ubiquitous in our environment and are found in many tissues in the body, especially fat. All of these xenobiotics pass through the placenta to reach the fetus. The perinatal period of the rat is a good model for the study of neuroendocrine differentiation since it is comparable to the first trimester of pregnancy in the human. The neuroendocrine effects of such xenobiotics are particularly dangerous because of the long delay between exposure and the onset of symptoms as shown in rat models. The existence of causal relationships in the human would be exceedingly difficult to discern unless animal work suggested a working hypothesis. The project is intended to examine the effects of various pesticides and chemically related agents on developing neuro-endocrine reproductive processes in the rat. Long term fertility effects of washing neonatal rats with phisohex are in progress. Hexachlorophene has a chemical structure similar to DDT and diethylstilbestrol which induce infertility when given to neonates. Hexachlorophene has been shown to produce neural lesions in newborn humans and rats. Preliminary data has shown that the cytoplasmic estradiol binding capacity is reduced in the 22 and 30 day old rats treated as neonates with estradiol benzoate. This study suggests that the reduced end organ (uterine) responsiveness described in neonatally treated animals with persistent estrus and polycystic ovaries, may not be the result of an absence of ovarian cycles, but the direct result of a neonatally induced and organ defect. Compounds including Dieldrin, Aldrin, Kepone, Mirex, Carbaryl, Aroclars, and several

Cannabinoids are being tested for potential induction of infertility through alteration of CNS mechanisms controlling sexual differentiation. Female rats washed with hexachlorophene exhibited normal puberty, estrous cycle and fertility, however, several males developed prostatic cysts and fertility was significantly impaired.

The perinatal effects of pesticides and other chemical agents have been investigated, focusing on functional impairments and behavioral abnormalities. Cyclophosphamide (30 mg/kg) strongly hindered mice in their ability to negotiate a rotating rod, and reduced brain weights significantly. Bidrin exposure *in utero* reduced locomotor activity in offspring of mice at seven weeks of age. *In utero* exposure to paraquat similarly affected the ability of mice to negotiate a water maze. Using fluroethyl-induced seizure threshold as an indicator of nervous system development, the deleterious effect of perinatal exposure to paraquat was demonstrated. Finally, PBB exposures were shown to cause birth abnormalities, behavioral abnormalities, and overt toxicities at the 50 to 100 ppm levels in the diet. These results indicate the sensitivity of organisms to these chemicals in the preweanling stage.

Certain chlorinated hydrocarbon pesticides have been shown to have estrogenic properties (e.g., DDT). The effect of these compounds on uterine enzymes and estrogen receptors is under investigation. The induction of ornithine decarboxylan (ODC) proved to be a useful assay for rapid estimation of "estrogenicity." Since ODC is presumed to be involved in cellular regulation, these effects could elicit certain subtle biochemical toxicities.

The effect of a number of pesticides on the electrical conductivity of membranes has been carried out in order to establish the main features of the molecular mechanism of action on charge transport through lipid membranes. This study has revealed that PCBT is sensitive to light and the equilibration time of PCBT between water phase and membrane is extremely long. While these findings have not been previously mentioned by other investigators, neglect of these effects could lead to erroneous conclusions. Using a technique to distinguish between the surface and internal characteristics of PCB treated membranes, the nature of surface adsorption and disassociation-recombination processes has been clarified. The technique has proven to be of value in identifying and characterizing the charge transport mechanisms involving other pesticides.

A study is underway to provide for rational design of specific insecticides and antimycotics through a basic understanding of the mechanisms of their action. It is felt that a better understanding of chitin synthesis might allow for the production of insecticide which react specifically on chitin and which would not be harmful to other organisms. During the past year, work on developing a satisfactory system for the *in vitro* study of insect chitin biosynthesis has gotten underway and has now been developed with Manduca tissue which deposits chitin at a known rate *in vivo*. The current *in vitro* system achieves chitin synthesis approaching 300 percent of the known *in vivo* rate.

Another approach to the design of selective insecticides for safer and more efficient control of insect pests of agricultural and public health importance involves basic enzyme studies. Moreover, it is thought that insects will prove to be excellent models for studying certain aspects of microsomal enzyme action and will provide information which can be applied directly to mammalian systems. Studies on the microsomal enzyme induction investigations have shown that the reaction leading to the p-hydroxylation of aniline differs in many ways (PH optimum and other assay conditions, sensitivity to inhibitors, K_m and etc.) from that effecting N-demethylation or epoxidation.

The effects of insecticides on the active and passive transport of cations through membranes is under study using frog skin, lobster giant axons and erythrocytes as models. Initial studies show the erythrocyte membrane to be extremely insecticide sensitive. Active sodium efflux from human erythrocytes was significantly inhibited at concentrations as low as 10^{-5} MDDT of hepachlor. These results indicate the possibility that some of the symptoms of acute insecticide poisoning may be due to this effect.

The applicability of cell culture techniques to pesticide binding and uptake studies are under investigation. The effects of culture conditions on these parameters, on the chemical stabilities of the compounds being studied and on cellular transport of these chemicals is being investigated. A number of insecticides have been screened for these effects. The data indicate that these procedures may be valid for initial biological testing in pesticide safety evaluation.

Radiolabeled glycosidic derivatives of appropriate metabolic products of selected pesticides are being prepared to investigate the metabolism of these compounds. Research on the behavior of conjugated materials in mammals has, in the past been inhibited by the difficulties encountered in preparation of radiolabeled compounds. Efforts so far have resulted in some modest successes mainly directed toward improving yields of synthetic products and not in developing entirely new synthetic routes. The significance of this phase of the work is in facilitating research on the mammalian metabolism of glycosides by improving the methods of radiosynthesis of such compounds. It is important to understand the behavior of plant conjugates of pesticides and pesticide metabolites in mammalian systems because of the likelihood of such materials appearing as minor components in the diet of animals, including man. If such conjugates are hydrolyzed in mammalian systems releasing a toxic aglycone, the significance is readily apparent. So far, in this research it appears that hydrolysis to the aglycone is a relatively minor occurrence. Direct oxidation of the glucose moiety of the administered conjugate to the glucuronide appears to occur.

A molecular mechanism of action study of anticholinesterase, some chlorinated hydrocarbons and the pyrethoids is focusing on a neural sodium pump mechanism and on actylcholinesterase function and activity. Housefly acetylcholinesterase has been isolated and characterized. It has been determined that a mutant enzyme binds to acetylcholinesterases with much less affinity than the normal form. Large differences in amino acid content of these enzymes were found. The phenomenon is under continued

investigation. Pyrethoids and DDT are possible sodium gate blockers and the techniques to study the process is under development.

Insecticide metabolism studies have been carried out on the chemical form of the sulfur bound to the macromolecules of rat hepatic microsomes incubated with carbon disulfide (CS_2) in the presence of NADPH. Approximately 45 percent of the labeled sulfur bound to the microsomes was released in the form which appears to be identical to thiocyanate. On the basis of these results it is proposed that a portion of the sulfur released in the microsomal metabolism of CS_2 reacts with the sulfhydryl groups of cysteine residues in the microsomal proteins to form a hydrodisulfide. Studies on the metabolism of parathion indicates that this material is metabolized by the reconstituted mixed function oxidase enzyme system to diethyl phosphoric acid, a product which, in previous studies using intact liver microsomes, had been thought to arise exclusively from the hydrolysis of paraoxon by a microsomal esterase. The data suggests that all three of the products of the metabolism of parathion by the reconstituted mixed function oxidase enzyme system; namely, paraoxon, diethyl phosphorothioric acid and diethyl phosphoric acid are formed non enzymatically by the breakdown by different pathways of a common enzymatically formed intermediate.

The effects of pesticides and other environmental chemicals on the functional development of liver, kidney, and lung is being undertaken to evaluate the mechanisms for age differences in toxic expression and to develop methods for assessing functional toxicities. Paraquat is less toxic to 21 day old rats than it is to adult or newborn rats, emphasizing the importance of its renal excretion. It was found that renal cortical slices from 21 day old rats could accumulate paraquat to a greater extent than tissue from adult or newborn rats. Although paraquat was not observed to be hepatotoxic, a reduction in hepatic concentration of glutathione (GSH) was observed. Following treatment to decrease hepatic GSH peroxidase activity, however, paraquat treatment resulted in significant liver damage. This suggests the removal of an important detoxification mechanism.

Studies to ascertain whether or not the rate limiting step in the excretion of drugs by liver is age dependent, were undertaken. Although it had been determined that hepatic excretory function in young rats is low compared with the adult, the mechanism of maturation was unclear. Studies on the excretory ability of young and adult rats suggest that, in adult rats, secretion of drugs from liver into bile is the rate limiting factor in excretion whereas in young rats hepatic uptake may be the major controlling factor.

Living organisms accumulate chlorinated hydrocarbons like DDT to levels far exceeding those in their environment. In part this arises because these compounds are excreted poorly. Therefore, a project was designed to examine the renal processes controlling DDT excretion and to use the information gained to develop methods capable of increasing DDT excretion, thus reducing the content in the organism. Major emphasis in this study is placed on the polar metabolite of DDT, DDA, the predominate form excreted in the urine of most animals. Results to date indicate that the PSP-PAH transport system is important in both mammals and fish. While

the events at the peritubular membrane are very similar in both (shown by the *in vitro* studies), fundamental differences are seen *in vivo*. The presence of net reabsorption in the rat suggests that drugs like probenecid or mersalyl, may be used to increase net DDA excretion. However, the magnitude of this effect and thus our ability to speed elimination may be severely compromised by the extensive and avid binding of DDA to plasma macromolecules.

Behavioral, biochemical and histopathological observations following exposure to pesticides is being correlated. Treatment with p,p'DDT mirex on low and high activity groups of mice have demonstrated significant behavioral modifications. Mirex initially suppresses activity and then produces marked hyperactivity prior to other symptoms of poisoning. DDT treatment effects the activity of hypo- and hyperactive mice differently and no overall trend has yet appeared. Hepatic cytochrome P-450 activity was significantly increased by treatment with both insecticides, and was found to be dose related. Another hepatic enzyme, NADPH cytochrome *c* reductase showed a marked increase post mirex treatment, but no increase post DDT treatment. Liver glycogen levels also appeared to decline after treatment with either pesticide.

Insecticide resistance is widely reported in invertebrates. Few such phenomena are known in vertebrate populations. One well documented vertebrate population resistant to organochlorine insecticides is the mosquito fish, *Gambusia affinis*. Physiological differences between resistant and susceptible *Gambusia* include membrane barriers and effective blood-brain barriers to organochlorines, higher liver lipid content, metabolic differences and a generalized CNS insensitivity to these compounds. It has been found that enzyme activities of the susceptible and resistant fish vary differently over the year and, surprisingly microsomal oxidase activity was *not* increased by raising pollutant levels in the water, although DDT itself did induce mixed function oxidase activity in both resistant and susceptible populations. Specific enzyme inductions, however, showed some differences between resistant and susceptible fish. These are undergoing further investigation as possibly relevant to resistance mechanisms.

Isolation of housefly cytochrome P-450 has confirmed that insect resistance mechanisms are similar to mammals. It has been determined that cytochrome P-450 exists in multiple forms and the enzymes are similarly inducible.

The effects of the organophosphorous pesticides on avian embryonic development is being studied, and in particular, to explore further the teratogenic action of azodrin and its interaction with nicotinamide and with antagonists of nicotinamide which are known to provoke similar embryonic abnormalities in avian embryos. Azodrin, in addition to its anti-cholinesterase activity is a potent teratogen. In chicken embryos at very low levels it causes a hundred percent of treated embryos to develop a severe form of micromelia with associated defects. This syndrome is one of the most common responses to a variety of specific vitamin antagonist and maternal nutritional deficiencies in chickens, as well as other vertebrate embryos, and also results from a variety of single gene mutations in all well studied

vertebrates including man. Therefore, it provides a useful model for studies of the genesis of this syndrome, in particular because its action during the entire embryonic period is accessible to study. It was found that azodrin administered by yoke sack injection causes striking morphological defects in chicken and Bobwhite quail embryos. Injection of chicken embryos at Hamburger and Hamilton stages 18 to 20 causes the most severe deformities. Altering the time of injection of azodrin or decreasing the level injected per egg alters the proportion of embryos effected as well as the severity of expression of one or more of the several defects. Azodrin was also found to depress acetylcholinesterase levels, decreasing hematocrit levels and altering the fluid balance of the chick embryo.

Organics

The mechanism of co-carcinogenicity of cyclopropenes is being studied by co-treatment of animals with sterculic acid and aflatoxin B_1. (Sterculic acid is a naturally occurring fatty acid in cottonseed oil.) Trout treated with this cylopropene fatty acid developed liver cancers and the effects of aflatoxin were potentiated. The hydrocarbon dioctycyclopropene gave similar results, indicating that the acid moiety is not essential. Metabolism of cyclopropene fatty acids is being investigated by C^{14} labeling. This study takes on particular importance in light of a recent petition for the use of cottonseed kernels as a food additive in baked goods, candies and smoked foods.

Polybrominated biphenyls (PBBs), a commerical flame retardant mixture, are persistant environmental contaminants with marked effects on hepatic tissue. In mice, liver weights and mixed function oxidase (MFO) activity increase significantly following a single acute exposure to PBBs. Neonate rats exposed to PBBs responded differently from adults showing an age dependent susceptibility in MFO activity patterns post PBB dosage. Therefore, the toxicity resulting from metabolites is not predictable from current available data. Further information on the process is being pursued.

The injurious effects of exposure to a related compound, polychlorinated biphenyls (PCBs), is being studied in adult non human primates. Female rhesus monkeys chronically fed diets containing 2.5 and 5.0 ppm PCBs developed acne, erythema alopecia and swelling of the eyelids. Blood changes were noted and adipose tissue accumulated the compound. Within four months menstrual cycles were altered. Six females were bred after six months of PCB exposure of 5.0 ppm and also eight females on the 2.5 ppm PCB diet. One live infant was born to the high dose animals and five to the low dose. The infants showed a rapid increase in PCB tissue levels, attributed to consumption of PCB-containing mothers' milk. Within two months, overt signs of toxicity were observed in the infants. Three of the six died within eight months. The remaining three were weaned and

showed a marked physical impairment, but also showed early signs of behavioral and learning impairments. Male adult monkeys, in similar experiments, showed a resistance to the toxic effects of PCBs.

The effects of benzene on the hematopoietic system is being studied. The sensitivity of lymphocytic precursor cells to chronic and acute *in vivo* benzene exposure has been documented. A new assay for studying B-lymphocytic maturation has been used for this study. Monocytes were similarly inhibited. The characterization of these toxicities by determining the active metabolite is underway.

Biodegradability of phthalate esters, one of the largest groups of aromatic compounds manufactured, is under investigation emphasizing a diverse group of microorganisms. Many of the bacterial strains isolated with phthalate esters have been characterized. A proportionately large number of them are Gram-positive cocci, which are not sufficiently similar to describe the species, and to allow further classification than Micrococcus sp. The isolates show quite varied abilities to utilize phthalate esters as carbon sources for growth. Sensitive esterase assays (developed for diagnostic purposes) were arbitrarily considered to be unsuitable as determinants of phthalate esterase activity since the aromatic moiety provides the carbonyl function. Therefore, a substrate specific assay has been used to determine phthalate esterase activity by titration of protons released during hydrolysis. For the strains of phthalate-ester utilizers so far examined, it seems that enzymes for their dissimilation are inducible, intracellular and have variable specificity for the alcohol moiety. Specificity of the aromatic moiety has not been examined. Without exception, all of the strains of Pseudomonads examined oxidize protocatechuate but only after growth with a phthalate or *p*-hydroxybenzoate. This was not always apparent since protocatechuate was not well oxidized by washed cell suspensions of one strain, but extracts readily accomplished this. This appears to be a very unusual observation and may provide a suitable model for the study of uptake systems for aromatic compounds, a rather neglected area of study. A salt-dependent (i.e., synthetic sea-water) oxidation of *o*-phthalate, 4,5-dihydroxyphthalate, and protocatechuate by a marine bacterium, strain OP has been shown to occur but only using washed cell suspensions and not by extracts of the cells.

Natural Toxins

The chemical make-up and the biochemical and physiological actions of the toxin from the jellyfish *Stomolophus meleogris* are being studied to establish biological mechanisms of action. An inhibitor of Na^{++}, K^{+}-stimulated ATPase was identified and isolated from one filter fraction of the venom and a stimulation of sodium transport and of ATPase was purified from another. The cardiac-acting components of the toxin are now under investigation.

In a study of the neurotoxicity of Brazilian rattlesnake venom, it has been learned that potent toxicity arises from the synergistic effect of two

proteins. These proteins from a complex in solution, and it has been determined that complex formation greatly enhances toxicity. The site of toxic action is indicated to be at the neuromuscular junction as one of the toxic components binds six times as strongly to diaphramatic tissue as to liver tissue. These, and structural identification studies are continuing.

The dermonecrotoxin produced by the brown recluse spider is being characterized and the complement inactivating substance and hemolytic agent of the spider venom is also being investigated. An *in vitro* toxicity assay for the venom has been developed using fat cell lysis. Venom protein in quantities as little as 1.0 μg will lyse 10^5 fat cells harvested from the rat foot pad. This lysis is determined visually (about 85 percent of the cells are usually lysed) or by the determination of fatty acid esters released from the cells (again about 85 percent of the available product is released). Phospholipase C, used as a control, will release the same amount of lipid provided 20 μg of enzyme are used, i.e., the venom is 20 times more active.

Doses of venom equivalent to that delivered in a spider bite will reduce the complement content of the blood of living guinea pigs by 50 percent within 18 hours, and is taken as evidence that decomplementation may be a normal part of the immunopathologic response to brown spider venom. A single preliminary experiment utilizing thin layer chomatography for lipids has revealed that venom (150 μg) is itself lipid free but will attack lecithin (5 μg) and release lysolecithin and free fatty acids. Lysolecithins tend to be hemolytic and it may be that the phospholipase activity in venom is cytodestructive to solid tissue cells and/or to red cells depending upon the quantity, location and distribution of venom deposited by the spider in its bite. Restated, the necrotoxin and hemolysin may be identical substances. The complement destroying principles in venom may liberate C3a, C5a or other molecules which originate from complement. These molecules tend to be both leukotactic and to produce inflammatory reactions in tissue.

Aflatoxin, the toxin produced by the fungus *Aspergillus flavus* which grows on a number of food products is known as a potent liver carcinogen. However, it is suspected that metabolism of the compound to the active material is required to elicit toxicities. Therefore, a number of studies on aflatoxin metabolism are being sponsored.

Labeled aflatoxin B_1 was prepared and injected into chickens and the metabolic products extracted from liver, bile, intestine and urine. None of the major metabolites isolated in this manner were found to be as toxic as aflatoxin B_1.

The metabolism of aflatoxin B_1 to DNA--alkylating metabolites and to other metabolites by hepatic microsomes is being studied. In addition, the effects of varied physiological, endocrinological and nutritional states on *in vivo* DNA-damage and repair that may follow aflatoxin administration is being evaluated.

Studies were conducted on rats in which physiological conditions producing differences in aflatoxin B_1 metabolism for which parallel examples exist in the literature for human populations exposed to aflatoxin contamination were carried out. Sex differences in sensitivity to aflatoxin toxicity in both humans and rats have been shown following exposure to aflatoxin. Studies on the effects of species, sex, strain, age, nutritional, hormonal, physiological and environmental manipulations should provide explanation at the molecular level for differences in aflatoxin toxicity and carcinogenicity due to these factors. In studies relating to sex differences, a good correlationship has been shown between the extent of *in vitro* metabolic activation of aflatoxin B_1 and susceptibility to carcinogenesis in rat. The male rat compared to the female has greater *in vitro* hepatic microsome-catalyzed DNA binding activity, binds more of the aflatoxin B_1 metabolite to DNA *in vivo* and is known to be more susceptible to aflatoxin carcinogenesis.

Human liver metabolic studies on aflatoxin are being carried out using biopsy specimens. Although metabolic patterns are complex, the six major metabolic products are similar to those observed in the lower species. Two out of three human specimens evaluated, metabolize aflatoxin B_1 into substances mutagenic to the bacterial test strain. Metabolic patterns can be altered by treatment with drugs. However, as in animals, the isolatable metabolites appear to be less toxic than the parent compound, indicating the elusive nature of the "ultimate carcinogen."

Molecular studies on the interaction of aflatoxin with DNA have demonstrated a strong binding between DNA and certain aflatoxin metabolites. By displacement studies, it was determined that aflatoxin B_1 interacts with adenine residues in DNA.

Other fungal toxins similarly present a hazard to farm animals and humans due to possible contamination of food. Ochratoxin was found to accumulate in liver and kidney, binding with proteins and subcellular organelles. The associated mechanism of toxicity may be elucidated by this observation. The compound was also determined to cause prenatal mortality when pregnant mice were treated during certain periods of gestation. In mice, the compound is also teratogenic.

Rubratoxin B also caused increased levels of prenatal mortality and teratogenicity. The compound's metabolism in the endoplasmic reticulum was established. In mice, the mitochondrial electron transport process appeared to be inhibited. Based on the known inhibition of the system by sodium azide and potassium cyanide, the principal site of action of rubratoxin on this system appears to be between cytochrome C, or C and the termination of electron flow.

Biomechanisms

The metabolism, disposition and interaction of water borne pollutants by fish is being determined to learn more about factors governing persistence

of residues in edible fish, to identify the metabolites of selected water pollutants and to determine factors and mechanisms of biotransformation and toxicities in aquatic species. An important lampricide "Bayen 73" was determined to be concentrated in the bile of rainbow trout and excreted by glucuronide conjugation to a water soluble product. The half life in various tissues ranged from 13 to 15 hours and indicated a low persistence potential. Carbaryl metabolism in rainbow trout indicated four major metabolites similar to those reported in mammals and indicating that bile analysis may be useful in monitoring fish for exposure to the compound.

A model of vapor uptake and excretion in man is being developed mathematically. A 10 compartment model for pharmocokinetic calculations has given more flexibility and accuracy to these computations. The new model has been verified by comparison with previous observations on benzene and methylene chloride inhalation. The model shows a striking effect of length of exposure on the fraction of material being exhaled and being metabolized. (The shorter the exposure, the greater the amount of material exhaled.) This confirms observations on benzene inhalation. Other important factors were alveolar ventilation and blood/gas partition coefficients.

The mechanism of lead toxicity to the immature brain is being investigated. The hypothesis is that lead primarily affects the maturing mitochondrial respiratory chain. Rat pups receiving lead carbonate in feed showed a loss of control with NAD-linked respiration and an increase in cytochrome oxidase activity. There was a progressive inhibition of ADP-dependent respiration with NAD-linked respiration and an increase in cytochrome oxidase activity. These observations precede gross and histopathological observations of toxicities. Work is continuing to establish age/susceptibility relationships.

The mechanism of lead induced brain capillary damage is being studied to establish whether or not these changes are related to the brain edema characterizing acute lead encephalopathy. By isolations of brain capillaries from immature animals, biochemical changes due to lead exposure can be determined. A biochemical index of diffentiation has been found and will permit study of the effect of chronic lead exposure on brain capillary development. Furthermore, methods have been developed to study the transport properties and membrane functions of brain capillary cells. It has been found that potassium uptake, which remains constant in the brain despite wide fluctuations in plasma levels, is greatly enhanced in lead poisoned animals.

The effect of carbon tetrachloride pretreatment on the pancreas has been noted as a sidelight of hepatic studies. The reduction in bile flow was found to be accompanied by an increase in flow of pancreatic fluid. By bile duct cannulation, it was determined that these alterations in flow rates accompanied all chlorinated hydrocarbons tested. This observation is significant in view of past data indicating that no general pancreatic damage results from exposure to carbon tetrachloride except for an occasional pancreatitis or fatty degeneration. The mechanism of this stimulation is now under investigation.

Techniques for the use of isolated enzyme systems for water purification are being researched. Candidates include arylamidase induced from Pseudomonas striata, organophosphorous hydrolyzing enzymes from several bacterial strains and nitrate/nitrite reductions linked to a cellular matrix. Systems for stabilizing these enzymes for potential use in drinking water plants are under development.

Mechanisms for recovery and repair of muscle and nerve tissue after exposure to organophosphate (OP) insecticides have been found to be proportional in rate to the degree of initial inhibition. Acetylcholinesterase (ACH) activity in cycloheximide treated cells reduces ACH within, but not outside of cells. Rapid cellular production of new ACH and a net increase in protein following treatment was also observed. The latter effect is postulated to result from an inhibition of protein degradation by OP's.

The role of membrane alteration in causing untoward effects subsequent to exposure to pollutants has been determined with certain species and insecticides. The basis of DDT-induced egg shell thinning was determined to be DDE interaction with calcium activated ATPase, an enzyme in the shell gland mucosa. Minute oral doses of crude oil given to young ducks and gulls indicates significant impairment of osmoregulation. In fish DDT has been found to effect both osmoregulation and intestinal absorption of amino acids.

Interactions

Biochemical information on the interactions of biodegradable organophosphorous (OP) insecticides with vital macromolecules is being sought. A major discrepancy between in vitro and in vivo OP metabolism data has been discovered. In vivo hepatic disposition of paraoxon is dominated by oxidative deethylation rather than by hydrolysis. This runs contrary to 20 years of thought on OP metabolism based on in vitro studies and inadequate chromatographic resolution of metabolic products. This finding will add greatly to the understanding of various OP toxicological phenomena.

The effects of piperonyl butoxide (PB), a widely used pesticidal synergist on microsomal enzyme induction and on the metabolism of carcinogens is under study. The compound acts by inhibition of pesticide metabolism and has been found to reduce the lethality and pathologic effects of dimethylnitrosamine (DMN) and methylcholanthrene (MC). The acute (LD_{50}) toxicity of DMN was unchanged by PB pretreatment. These results indicate the importance of metabolism in the carcinogenic process.

The theory that environmental chemicals at normal exposure levels may enhance or reduce the toxicity of compounds by metabolic changes is being pursued. Two chemical models for this phenomenon are being developed. Hepatic centrilobular necrosis induced by carbon tetrachloride and toxic effects on isolated hepatocytes by this compound are variable in degree, based on pretreatment with metabolism-altering chemicals. Similarly, allyl

alcohol hepatotoxicity is under similar investigation. The two model compounds represent important chemical classes of industrial chemicals.

A study of the protective effect of chloramphenicol (CAP) on carbon tetrachloride exposure, focused on involvement of microsomes or mitochondrial membranes. It was found that the activity of sulfite oxidase, a parameter of mitochondrial outer membranes, increased as mitochondrial damage increased. A concomitant 50 percent decrease in monoamine oxidase, an enzyme bound to the outer membrane, was also noted. In the endoplasmic reticulum (ER), CAP protection is observed only when administered within five to seven hours of carbon tetrachloride dosing. Loss of protein synthesis was then partially prevented. The biochemical events associated with this process are now being investigated.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement

During the past year the Associate Director for Genetics has continued old programs developed as Chief of the Environmental Mutagenesis Branch (1972-1976) and has developed new projects in the area of environmental mutagenesis in the following areas (1) International Programs (2) National Programs (3) Training Courses (4) Committees (5) Collaborative Studies and (6) Collaborative Research Programs, and (7) Public Lectures.

International Programs

Under the US-USSR Environmental Protection Agreement the Fourth Joint Workshop on Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis was held on April 12-14, 1977 in San Francisco. The program discussed techniques for automated karyotype analysis, sister chromatid exchange, assays for DNA repair, somatic cell techniques for population monitoring and germ cell techniques. Under the US-USSR Environmental Health Agreement, steps have been taken to start a new program of collaboration in human population monitoring and epidemiology with the Institute of Medical Genetics, Moscow.

Under the US-Japan Cooperative Medical Sciences program the joint Environmental Panels organized a Fifth Joint Conference on Approaches to the Prevention of Environmental Cancer and Genetic Diseases which was held in Nikko, Japan on August 3-5, 1976. Major emphasis was placed on development of methodology for screening populations for environmentally induced cancer and to identify cancer families; the utility of short-term tests for identifying potential mutagens and carcinogens; and in a final session new techniques for detecting somatic mutations in red blood cells and in human cells in culture were compared. Under the US-Japan Comparative Cancer Research Program the Associate Director for Genetics worked with Dr. H. Gelboin, NCI and Dr. P. T'so, Johns Hopkins University to develop a program for the joint conference on "Polycyclic Aromatic Hydrocarbon Carcinogenesis, Biochemistry and Environment" which was held on January 23-26, 1977 in New Orleans.

The first organization meeting of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) was organized by the Office of the Associate Director for Genetics and the meeting was held at NIEHS on January 16-19, 1977. This group met to identify major problem areas as well as to identify committee chairmen to send the various working groups. An agenda was also planned for the next meeting of the Executive Committee with the heads of various sub-committees in Lausanne, Switzerland on September 25-30, 1977.

National Programs

(1) Subcommittee on Environmental Mutagenesis (SEM) - Monthly meetings chaired by the Associate Director for Genetics have been held to review current problems in environmental mutagenesis; a detail list of meetings and minutes have been submitted to the parent committee (DHEW Committee to Coordinate Toxicology and Related Matters).

In addition the final draft of the working group on "Approaches to Determining the Mutagenic Properties of Chemicals; Risk to Future Generations" has been prepared and submitted to the parent Committee.

(2) Plans have been developed for a conference on Comparative Chemical Mutagenesis to be held at the Sheraton Inn, Raleigh, NC from October 30 - November 5, 1977. In this conference the mutagenicity data on 20 selected chemicals will be tabulated and compared with regard to effects on experimental organisms and on man (when data are available) to learn how to extrapolate data for experimental organisms to the human population. In preparation for this conference two preliminary workshops were held:

(a) The first workshop was held to review the position papers being prepared by the first working groups on the effects of the chemicals selected for study on different laboratory organisms. This workshop was held immedidately after the Annual Environmental Mutagen Society Meeting in Colorado Springs on February 18-19, 1977.

(b) The second workshop to review the position papers being prepared by the second working groups on qualitative and quantitative comparisons of the effects of each of the 20 chemicals selected for study was held on July 12, 1977 in Edinburgh during the Second International Conference on Environmental Mutagens.

A small workshop consisting of about 12 experts in the field of mammalian genetics and cytogenetics was developed under joint NIEHS, ERDA and NTCR sponsorship to evaluate the testing procedures used in the heritable translocation assay in rodents. This meeting which was held at Stanford Research Institute, Menlo Park, CA has drafted a report to identify the important problem areas and to develop collaborative studies between research groups using this assay procedure.

A workshop on Higher Plant Systems as Monitors of Environmental Mutagens is being planned for January 1978. The purpose of this workshop is to identify and develop additional assay systems to complement the Tradescantia assay for monitoring air pollutants as well as to develop other approaches for evaluation of air and water pollutants.

Plans have also been made to develop a workshop to discuss the Development of Guidelines for Evaluation of Mutagenicity Screening Data in collaboration with CIIT in August 11-12, 1977.

Training Courses in Environmental Mutagenesis

This office has participated in the development of training courses at the University of Texas Medical School, Galveston which was held on September 19-24, 1976 and the workshop on Mutagenesis and Predictive Carcinogenesis which was held in Edmonton, Canada at the University of Alberta on May 1-6, 1977. In addition preparations are being made for other courses to be held in Cairo, Egypt and Mexico City, Mexico in the beginning of FY 1978.

Committees

The Associate Director for Genetics has also participated as a Consultant to the EPA Environmental Health Advisory Committee in the development of criteria and guidelines for mutagenicity testing of pesticides. In addition, as a member of a new FDA Task Group a similar effort has been started to develop criteria and guidelines for mutagenicity testing of food additives with special emphasis on GRAS list chemicals. As a member of the NCI in vitro mutagenicity tests subcommittee new contracts have been prepared to develop and refine short-term tests for carcinogenicity.

Collaborative Studies

A new collaborative study has been developed to evaluate and compare various tests for mutagenicity in blind tests on about 45 compounds consisting of pairs of carcinogens and noncarcinogenic structural analogs as well as selected chemical mutagens from different chemical classes. This study has been developed in cooperation with the Imperial Chemical Industries, the International Association of Environmental Mutagen Societies and NIEHS. The study will be divided into two phases consisting of (1) short-term tests for mutagenicity - Phase I and (2) tests designed for verification and quantitation of mutagenic activity - Phase II. The study will involve collaboration between about 43 scientists in different laboratories all over the world.

Collaborative Research Programs

The Associate Director for Genetics has continued the analysis of the mutagenic activity of various classes of chemical carcinogens in wild-type and excision-repair deficient two-component heterokaryons of Neurospora crassa. This work is being performed with the collaboration of the University of Illinois, Normal and the Miles Laboratory, Elkhart, Indiana (see LEM report). These studies are designed to determine whether chemicals are carcinogens because they produce some characteristic mutagenic lesion. Previous studies have shown that in (wildtype) Neurospora chemical carcinogens are base-pair substitution mutagens, whereas in (excision repair deficient) Salmonella they are frame-shift mutagens. Current data in Neurospora where comparisons have been made between wild-type and excision-repair deficient strains shows that base-pair substitution mutagens in wild-type strains are converted to frame shift mutagens in excision-repair deficient strains. More extensive data are being sought by testing chemical carcinogens from different chemical classes to determine the generality of these conclusions.

In another collaborative research program a higher plant system (Tradescantia) is being developed and used in a mobile laboratory to monitor airborne pollutants in collaboration with the Brookhaven National Laboratory (BNL) and NERC-EPA. Plans have been made to monitor the ambient air in various high cancer incidence counties around the country during the next year. Significant increases in mutation frequencies have been found in four of the five cities monitored during the first half of FY 77. These are exploratory studies to evaluate the general utility of this assay system as well as to determine the type of air pollutants that will give a positive response with this assay system. The group at NERC, EPA has the responsibility for providing data for selection of sites as well as providing the physical monitoring data concerning the spectrum of pollutants (nonorganic and organic) in ambient air at each site. The preparation of plant material and evaluation of mutagenic activity is carried out at BNL.

In another area attempts are being made to evaluate the ability of an assay system which has been developed in Neurospora crassa to screen chemicals for nondisjunction. Significant progress has been made in both validating this procedure as well as evaluating its general utility as a prescreen.

Lectures

The Associate Director for Genetics has given a series of invited lectures in connection with various meetings, workshops, conferences and symposia on current problems in environmental mutagenesis as follows:

1. "Perspectives on the Mutagenicity of Chemical Carcinogens" 6th Annual Meeting of the European Environmental Mutagen Society, Gernrode, GDR, September 27 - October 1, 1976

2. "Strengths and Weaknesses of Microbial Test Results for Predicting Human Response" - NCTR/NIH Conference on the Status of Predictive Tools in Application to Safety Evaluation: Present and Future - Little Rock, Arkansas, November 17-19, 1976

3. "Short-term Tests for Mutagenicity to Detect Carcinogenic Potential of Environmental Chemicals" - Environmental Carcinogenesis Conference, Houston, TX, 12-13 January 1977.

4. "Testing the Genetic Potential of Environmental Chemicals" Seminar, February 11, 1977, Chapel Hill, NC, UNC

5. "General Perspectives and Utility of In Vitro Assays" Symposium on Mutagenesis, New Brunswick, NJ, February 24, 1977

6. "Import of Recent Discoveries in Chemical Carcinogens" Mini Symposium on Chemical Carcinogens, Chapel Hill, NC, UNC, March 24-25, 1977

7. "Effective Utilization of Newly Developed In Vitro Techniques for Evaluating Mutagenic and Carcinogenic Potential of

Environmental Chemicals" - Colloquium on Environmental Toxicology, University of Wisconsin-Madison, March 29, 1977

8. "Effective Utilization of Short-Term Tests for Mutagenicity to Evaluate Environmental Chemicals" - All Union Congress on Genetics, Leningrad, May 16-20, 1977

HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

This office consists of six professionals and support personnel concerned with the evaluation of environmental health hazards due mainly to chemical agents. An effort initiated earlier to pull together toxicologic information on organ systems and their diseases in humans with environmental or occupational chemical exposures was continued. It received its impetus when the cause of the Legionnaires' disease was suspected to be inadvertent exposure to a chemical agent. Many difficulties have been encountered in carrying out this compilation, particularly the utilization of anecdotal reports on cause/effect relationships, suggesting that the compilation of data needed experimental proof of cause-effect relationships.

This office collaborates with other governmental agencies through the Office of the Assistant Secretary for Health, DHEW, to evaluate or critically review Environmental Impact Statements or Effluent Limitation Guidelines. This activity has been centered on Environmental Protection Agency (EPA) documents, but also reviewed drafts and documents of other departments.

During FY 77 this office supplied most of the members of a Subcommittee on Human Health Consequences Due to Lead Exposure from Automobile Emissions sponsored by the DHEW Committee to Coordinate Toxicology and Related Programs. The document prepared by this Subcommittee was widely distributed and comments received from experts were incorporated into the final version to be published in ENVIRONMENTAL HEALTH PERSPECTIVES (EHP). The contents served as background information to the EPA in their activity towards setting a standard. Subsequently, members of this office served for a considerable time as consultants to the EPA in preparation of the final EPA document.

The DHEW Committee to Coordinate Toxicology and Related Programs created a Toxicology Information Subcommittee of which Dr. Damstra of this office is a member. It also created a DHEW Laboratory Chemical Carcinogens Safety Standards Subcommittee on which the Director, OHHA, is a member helping in the determination of safety rules and reviewing the monographs written on safe handling of individual carcinogens.

Other collaborative efforts served to assess the potential health hazards from the use of ethylene oxide as a chemosterilant in hospital sterilization of instruments and equipment, where the Director of the Office of Health Hazard Assessment (OHHA) served as Chairman of an HEW Subcommittee. The Director, OHHA, also served on a Task Force on the Health Implications of Nitrilotriacetate, created by the International Joint Commission, Great Lakes Research Advisory Board, Windsor, Canada.

With the activation of the Toxic Substances Control Act in 1977, this Institute was asked to supply a member for the Federal Committee for Selection of Substances for Testing in order to help the EPA carry out their newly assigned mandate. This Office supplied both member and alternate for this committee, which has to prepare a list of no more than 50 of the most important hazardous chemicals for which further toxicological testing is needed.

A Committee, composed of one member from each branch or laboratory of the Institute under the chairmanship of the Director, OHHA, prepared a priority list for in-house research on toxic chemicals.

Since the designation of NIEHS as a WHO-Collaborating Center, this office has supplied information, expertise, and consultation to WHO. For a meeting of a Scientific Group on Environmental Health Criteria held at Geneva, November 22-26, two papers were submitted on request by WHO, on "Health Risk Assessment from Experimental Data (Low dose extrapolation; transposition of animal data to man)" and on "Health Risk Assessment from Epidemiological Data". The Director, OHHA, was invited to participate at that conference. For another meeting, the WHO requested a paper on "Guidelines for Establishment of National Environmental Quality Standards" which was submitted to WHO. Dr. Piver of this office was requested by WHO to spend three weeks from May 8-27, 1977, in Geneva as Consultant to WHO to write a report on "Environmental Health Problems Associated with the Manufacture of Synthetic Organic Chemicals". This service to WHO overlaps with the functions of this office to be concerned also with the potential health hazards of new uses of chemicals and of technological developments which can already be forecast.

Additional collaborative services are rendered to WHO in critically reviewing their Environmental Health Criteria documents as well as separately printed summary statements. This WHO activity is so closely aligned with the program of this office that the collaboration is mutually beneficial.

Dr. Martin of this Office attended the International Conference on Environmental Arsenic, an NIEHS-WHO-sponsored meeting.

Following the WHO/NIEHS-sponsored Symposium on Potential Environmental Health Hazards from Technological Developments in Rubber and Plastics Industries, held at this Institute in March 1976, a conference was held this FY, inviting participation of members of WHO on the topic, "Comparative Metabolism and Toxicity of Vinyl Chloride Related Compounds". This conference held at NIH, Bethesda, Maryland, in May 1977, brought together most of the experts on this topic from the USA, Europe, and Japan and showed that toxicity of these compounds varied considerably and could not readily be predicted from chemical structure alone. Personnel of this Office was largely responsible for preparing the conference and carrying it through. The proceedings will shortly be published in EHP.

On request by the Office of the Assistant Secretary for Health, a literature search on ozone as a disinfectant of drinking water was carried out by this office which was presented at the Ozone/Chlorine Dioxide Oxidation Products of Organic Materials Workshop, held in November 1976.

This office frequently supplied staff to attend meeting sponsored by industry, universities, or governmental agencies when that was requested by the Director, NIEHS, or NIH. Thus Dr. Martin attended meetings of the American Society for Testing of Materials (ASTM), Medical Toxicology Subcommittee, as well as a Brookhaven National Laboratory Workshop on Sulfate Air Pollution.

Dr. Posner of this Office has been a member of the Federal Task Force on Inadvertent Modification of the Stratosphere (IMOS), an activity which will shortly be coming to an end.

The Director, OHHA, also serves on a Committee for the U. S. Department of Agriculture Expert Panel on Nitrites and Nitrosamines, to review the risks of nitrite and nitrate treatment of bacon and cured meat products, due to the formation of nitrosamines. As a member of the committee, he is concerned also about the environmental impact of recommendations changing the processing of such commodities without the use of nitrites and nitrates.

The Director, OHHA, was invited to appear before the Subcommittee on Energy and Power of the Committee on Interstate and Foreign Commerce of the U.S. House of Representatives to make a statement on hazards or potential hazards resulting from coal liquefaction.

An important issue confronting the Federal enforcement agencies is the question of a permissible carcinogenic risk to the general population which cannot be evaluated by the individual. Experimental data obtained in the laboratory on the adverse effects of a certain chemical come from a species which although comparable to the human is not similar, and the dose administered to a small group of animals has to be large to produce a significant effect. The extrapolation of such data from the laboratory to human experience is very difficult, and considerable efforts have been made by this office to detect factors which may allow better risk assessment. Knowledge on the biochemical and physiological as well as pathological level is looked for in attempts to clarify internal and external variables at play. Such information is disseminated to stimulate responses, be they rebuttals or confirmations.

The Director, OHHA, represents the NIEHS at the National Academy of Sciences Medical and Biological Effects of Environmental Pollutants (MBEEP) Committee, and this office helps in the review of criteria documents prepared by this body. He also served the American Physiological Society as Associate Editor of the HANDBOOK OF PHYSIOLOGY, Section 9, "Reactions to Environmental Agents". He also contributed a chapter on "Chemical Agents in Cigarette Smoke".

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20001-05 OHHA

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Variations in the Response of Rats to Porphyrogenic Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hans L. Falk	Assoc. Dir., OHHA	OHHA NIEHS
OTHER:	Sandra Jordan	Technician	OHHA NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective is to examine the variations in response of rats to porphyrogenic chemicals. Hexachlorobenzene was the chemical of choice, because it is an important fungicide and produces porphyria following ingestion for a few days. The porphyria reaches its peak a few months later and slowly declines. The pattern has been followed in individual rats. The existence of rats which do not respond by elimination of uro- or coproporphyrins in the urine has been detected. The animals were followed through their recovery period of 18 months after which time no evidence of porphyria remained. They were then given another porphyrogenic chemical, allyl-isopropylacetamide, (AIA) in the diet or by gavage to observe rapidly occurring and short-lived porphyria, resulting from this treatment. Variations in response of these rats to repeated application of this chemical were compared to their response to hexachlorobenzene. Explanations for the differences in response are being studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats were given hexachlorobenzene in the diet for a short time interval. The elimination of copro- and uroporphyrin in the urine was quantitated spectrophotometrically. Fluorescence was looked for in feces and tissue after necropsy. AIA was administered to the same rats after complete recovery from exposure to hexachlorobenzene to compare the response of individual rats to this chemical. AIA was given in the diet or by gavage as a suspension in oil.

MAJOR FINDINGS AND PROPOSED COURSE: The porphyria produced in these rats differed considerably in individual animals. In those animals which responded the maximal urinary elimination of uro- and coproporphyrins was reached in 8 to 9 months and declined to control levels in 18 months. However, in some animals no response was observed in urinary porphyrin excretion after feeding of hexachlorobenzene. The response to allyl-isopropylacetamide administration was rapid of very short duration and variable. Again, the animals that did not respond to hexachlorobenzene also did not respond to this chemical by urinary excretion of porphyrins. Even one of the "hexachlorobenzene responders" did not show uro- and coproporphyrins in the urine after AIA. An explanation for the lack of response will be looked for using AIA as the porphyrogenic chemical of choice. The chemical will be administered by gavage, so that variation in dietary intake can be eliminated. Urine and feces will be examined as well as tissue for evidence of porphyria. The inhibitory effect of pretreatment with phenobarbital on porphyrogenesis by AIA will be studied and extended to hexachlorobenzene to obtain information on spatial and quantitative parameters of these interactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Variation in human response to environmental chemicals has often been demonstrated. The very marked variation in response of inbred rats to porphyrogenic chemicals is studied to determine the degree of variation in response to porphyrogens of different chemical structure to be compared to the variations superimposed by pretreatment with common enzyme inducers, which are also encountered by human populations in their daily lives.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20002-05 OHHA

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Technology Forecasting and Technology Assessment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Warren T. Piver	Chemical Engineer	OHHA NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS
	Herbert S. Posner	Pharmacologist	OHHA NIEHS
	Terri Damstra	Biochemist	OHHA NIEHS
	Alec E. Martin	Visiting Scientist	OHHA NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The goals of this program are: (1) develop a methodology for technology forecasting and technology assessment for the chemical process industries and the energy production industries that would provide guidance in setting research priorities in environmental health and (2) develop environmental transport models and methods of parameter estimation for use in designing solid and liquid waste management practices.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: For both objectives, computer simulation will be the main tool. Trends in production, rates of substitution of products, and the impact of Federal legislation and programs on chemical and industrial developments can be modeled. This information can be coupled with available basic biomedical and toxicological data to provide preliminary environmental assessments and point out gaps in information. Environmental transport processes which comprise a major portion of the assessment process can be simulated. Actual experimental data will be required to validate the model and its transport properties, but additional research efforts are required to estimate these properties directly.

MAJOR FINDINGS AND PROPOSED COURSE: The major findings of this activity has been towards defining the scope of the problem. For example, as a result of Federal legislation, a large number of chemicals are being developed and marketed as fire retardant chemicals. In many instances the mechanisms of retardation and the nature of the combustion products are poorly understood. In addition, the toxicities of the chemical and its combustion products are poorly characterized. Federal energy programs have called for the development of large deposits of coal and oil shale for energy production. The environmental health impact of this type of development is poorly understood, particularly in the area of management of solid and liquid wastes from these processes. Efforts are currently underway to simulate the transport in solid waste disposal sites for the purpose of developing better waste management practices. This information and approach can be used in other solid waste management programs.

When coupled with considerations of possible relationships between chemical structure and biological activity, an ability to forecast in a reasonable fashion the rate of substitution of chemical products in the market place will be most useful in beginning to determine the types of biomedical research programs needed to assess the environmental health impact of commercialization of that product. This would be useful to NIEHS and the EPA Office of Toxic Substances. Initial efforts to include Institute personnel in the process of determining research priorities consistent with established and on-going laboratory programs resulted in a list of chemical that fulfilled these considerations and will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These activities will continue to provide necessary information about products and use patterns of chemicals, important environmental transport mechanisms, and development of methods to predict exposure levels for environmental chemicals. Such information can be used in planning research programs for the Institute.

PUBLICATIONS

1. Piver, W. T. Diffusion of monomers in polymers. Environ. Hlth. Perspec. (In press).

2. Piver, W. T.: Environmental transport and transformation of automotive emitted lead. Environ. Hlth. Perspec. (In press).

3. Piver, W. T.: Emission control devices, fuel additives, and fuel compositional changes. Environ. Hlth. Perspec. (In press).

4. Nelson, N., Van Duuren, B., Goldschmidt, B. M., Johnson, J., Moolenaar, R., Fisher, F., Weisburger, E., Piver, W. T., and Shaffer, C. B.: Final Report of NSF Workshop Panel to Select Organic Compounds Hazardous to the Environment, October 1975.

5. Hathaway, C. E., Bletzacker, R. W., Clarke, G. H., DeRis, J., Gross, D., Norris, J. M., Piver, W. T., Roux, H. J., and Vessel, E. D.: Assessing Fire Hazard -- Some Research Needs. Report of American Society of Testing Materials (ASTM) Subcommittee E39.10.01, Task Group 3 on Fire Research Needs, April 1977, Philadelphia, Pennsylvania.

6. Burger, E. J., Jr., Friedlander, S. K., Borg, D. C., and Piver, W. T.: Environmental forecasting and technology anticipation. Chapter 8. In Nelson, N. and Whittenberger, J. L. (Eds.): Report of Second Task Force for Research Planning in Environmental Health Sciences. (In press).

7. Royce, E., Gross, T., Zerbe, J. I., Cowan, A. M., Piver, W. T., Anderson, R. W., Ball, R., Owens, J., and Maust, E. E.: Conservation and recovery of materials and energy panel. In Federal Council for Science and Technology: A National Plan for Energy-Related Materials Research and Development. Part I. Near-Term Program. Prepared at the Request of the Committee on Materials (COMAT), ERDA Report 76-28, April 22, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20003-04 OHHA

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Preventive Surveillance of Environmental Chemicals for Toxic Potential

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert S. Posner	Pharmacologist	OHHA NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Two compound-groups are being followed: the relatively new aminimides and the vinyl halide-related compounds. New uses and further development of previously described uses of aminimides are being reported. Toxicity has not yet become a problem with aminimides. Clarification is beginning to develop of the relationship of structure to toxicity in the vinyl halide-related series. Efforts continue in an attempt to guide safe uses of methanol in the future because of the expected rather large increases in use and in new situations of rather general population and environmental exposure. National and international correspondence is being maintained. The first biochemically-oriented conference on paraquat toxicity has been held. It is hoped that this will hasten increased safety with regard to uses of paraquat. Lead air standards, the questioned continuing use of lead in vehicular fuels, and the screening for lead exposure and toxic responses to lead have been active areas with regards to lead this year. The IMOS Task Force appears to have completed its role, and research on short and longer term ultraviolet hazards is proceeding via EPA as the lead agency and on the basis of initial proposals developed by the Biological and Climatic Effects Research (BACER) subcommittee of IMOS and its subsequent Workshops.

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, consultations with appropriate individuals, consideration of known structure-activity relationships, attendance at meetings, preparation of reports, discussions with those who might assist in further laboratory or theoretical considerations, feedback and dissemination of the appropriate findings, and preparation of manuscripts for publication. Reviews of documents and manuscripts and response to letters as requested.

MAJOR FINDINGS AND PROPOSED COURSE:

Aminimide compounds: The literature is being followed for this interesting and relatively new series of chemicals as discussed in three earlier reports. Patents for the antibiotic activity of some long chain fatty acid derivatives have been granted. Herbicide activity continues to be reported. The preparation of aminimide copolymers that contain 2,4-D or 2,4,5-T structures provide polymers with varying hydrolysis rates for the release of 2,4-D or 2,4,5-T moieties. Other reports further describe the use of some aminimides as detergents, on textiles and in other uses that involve general population exposures. Many new aminimide compounds have been synthesized and their conversion products described.

Compounds with vinyl halide or precursor vinyl halide substructure: A report was presented at the NIEHS "Conference on Comparative Metabolism and Toxicity of Vinyl Chloride Related Compounds," May 2-4, 1977.

Methanol: Continued efforts are being made to advise those who want to make rather large use of methanol in the general environment of the biologic hazards. The testimony of an individual at a California Senate Committee hearing includes this remark: "...the excellent review on 'Biohazards of Methanol in Proposed New Uses' as prepared by Herbert Posner of the National Institute of Environmental Health Sciences...". A letter to the Chairman of the Committee unfortunately did not bring a transcript of the hearing. Correspondence with another individual brought a copy of a nearly 400 page translation, from German, on alternative fuels. A call from the Swedish Embassy for further information about methanol, because of earlier correspondence with Volvo, brought a copy of a recent symposium in exchange for this information. Hazards of methanol were reviewed at a "Workshop on the Assessment of Health Effects of Synthetic Fuels" at the Brookhaven National Laboratory, September 27-29, 1976.

Paraquat: A talk entitled "Overview of Paraquat Toxicity: Paraquat is not a Hit-and-run Poison" was presented at the "Conference on Biochemical Mechanisms of Paraquat Toxicity" at the University of Iowa, June 27-30, 1976.

Lead: There was participation on the "Subcommittee on Human Health Consequences Due to Lead Exposure from Automotive Emissions" of the DHEW Committee to Coordinate Toxicology and Related Programs, and further development of a chapter from

this report for publication in Environmental Health Perspectives. Two EPA meetings were attended with regard to development of air standards for lead, and the working and final documents are being reviewed. There was attendance at two meetings with the North Carolina Department of Health to explore setting up a screening program for lead exposure in the State. I participated in the NIEHS "Workshop on Planning a Pediatric Lead Screening Program in North Carolina," May 5, 1977, and presented the talk "Laboratory Methods of Assessing Total Body Lead Burden".

Outside activities: Several Workshops were attended concerning the research proposals of the Biologic and Climatic Effects Research Subgroup (BACER) of the Interagency Task Force on Inadvertent Modification of the Stratosphere (IMOS). The recommendations of the Task Force were presented by invitation at a Conference entitled, "A Ban on Aerosols?" in Zurich, Switzerland. Other outside activities are as above.

Other: I responded to the request of the Director of NIEHS to rewrite and refine the Recommendations of the Second NIEHS Task Force Report. There were reviews of NAS, EPA, NIOSH, and other documents as requested and response to letters on above issues.

PROPOSED COURSE: The new findings of biologic and toxicologic importance for the aminimide compounds will be followed as they appear. The vinyl halide related compound situation will be followed closely and a manuscript in preparation will be completed. Additional channels will be sought for appropriate consideration of the hazards of methanol so that, if methanol is to be used in some of the proposed situations, its hazard can be minimized. Paraquat and lead hazards will be monitored. Meeting attendance, participation on special projects, review of documents and manuscripts, and responses for information will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas where research is needed in order to prevent or reduce toxicologic hazard from environmental chemicals or from sources of physical stress (the latter as in the IMOS considerations). It is primarily a program of preventive and early-warning surveillance. It is linked closely to research dealing with the scope of the problem, mechanisms involved, and preventive measures. It also takes into consideration potential means of diagnosis and therapeutics where these might be possible.

PUBLICATIONS

Posner, H. S. and Falk, H. L.: Haloethylene and related compounds of industrial, environmental and medical significance. Environ. Hlth. Perspec. (in press) 1977.

Posner, H.S.: Indices of potential lead hazard. Environ. Hlth. Perspec. (in press) 1977.

Posner, H. S.: Recommendations of the Task Force on Inadvertent Modification of the Stratosphere. Gottlieb Duttweiler Institute for Economic and Social Studies (in press) 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20004-03 OHHA

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Surveillance of Potential Environmental Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terri Damstra	Biochemist	OHHA NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS
	Warren T. Piver	Chemical Engineer	OHHA NIEHS
	Herbert S. Posner	Pharmacologist	OHHA NIEHS
	Alec E. Martin	Visiting Scientist	OHHA NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The long-range objective of this project is to provide information on the target organ toxicity of environmental chemicals. Initial emphasis has been placed on identifying the associations between central and peripheral nervous system diseases and chronic low level exposures to environmental and occupational chemicals.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparation of an open-ended data file, which is continually updated via constant literature surveillance along with computer access to the NLM bibliographic data banks. Membership on committees; attendance at meetings; reviews of documents and manuscripts; preparation of reports and monographs; and consultations with scientists from other Government agencies, industry, and academia.

MAJOR FINDINGS AND PROPOSED COURSE: Human populations are exposed to a number of toxic agents which may affect the central and peripheral nervous system at the threshold concentration or at concentrations two to tenfold above the recommended level. These agents range from toxic gases, heavy metals, chlorinated hydrocarbons, organophosphorus insecticides, industrial solvents, and household products, to a large number of drugs such as tranquilizers, stimulants, hormones, and anaesthetics. Prenatal and neonatal exposures may have neurobehavioral effects which are not expressed until much later in the life span of the organism. The old and "stressed" organism may also exhibit enhanced susceptibility. A review on the target organ toxicity of lead has been completed. A compilation of agents whose main toxic effect is peripheral neuropathy includes many widely used commercial and industrial compounds. These may cause irreversible peripheral neuropathy at dose levels where other systems are not affected. Little is known about the mechanism of toxicity of these compounds. Continuing surveillance of these compounds is critical.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A compilation of associations between target organ toxicity and exposures to chemicals will aid the Institute in making a proper assessment of potential health hazards and in identifying the research programs necessary before such assessments can be made.

PUBLICATIONS

1. Damstra, Terri. Toxicological properties of lead. Environ. Hlth. Perspec. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20005-02 OHHA

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chronic Lead Exposure: Neurochemical Assessment of Developmental Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terri Damstra	Biochemist	OHHA NIEHS
OTHER:	Carole A. Kimmel	Senior Staff Fellow	LET NIEHS
	L. D. Grant	Associate Professor	UNC

COOPERATING UNITS (if any)

None

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range goals of this project are to elucidate the biochemical basis of lead toxicity in the developing central nervous system. The initial objectives are to determine whether chronic lead exposure is accompanied by alterations in the developmental patterns of the neuronal enzyme, Acetylcholinesterase, and the glial enzyme Butyrylcholinesterase.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats were chronically exposed to lead acetate via their drinking water (0, 5, 250 ppm) from weaning through mating, gestation, and lactation. The telencephalon, brainstem, and cerebellum of 7 and 30-day old male offspring were assayed for acetylcholinesterase and butyrylcholinesterase activity, and protein and DNA concentration, using standard biochemical techniques. Blood and bone lead concentrations were also determined.

MAJOR FINDINGS AND PROPOSED COURSE: Body and brain weights were slightly reduced in the offspring exposed to 250 ppm lead, but the differences were not significant. Regional brain DNA and protein concentrations did not vary between control and lead-treated animals. Acetylcholinesterase activity was significantly depressed in the cerebellum of both the 5 ppm and 250 ppm lead-treated animals at 7 days of age and in the cerebellum of the 250 ppm animals at 30 days of age. Butyrylcholinesterase activity was also depressed, but the differences were not significant. The above neurochemical parameters will be evaluated in conjunction with tissue lead concentrations and various developmental and behavioral assessments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developmental toxicity of lead due to chronic exposure is of great concern due to its wide-spread low-level contamination. Although lead influences the activity of several enzymes, no biochemical basis for lead toxicity in the central nervous system has been clearly established. An analysis of the above enzyme systems may help elucidate some of the factors involved in the toxic manifestations of lead in the nervous system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20006-01 OHHA

PERIOD COVERED
September 25, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Surveillance of Potential Environmental Health Hazards
(Medical and Epidemiological Aspects)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alec E. Martin	Visiting Scientist	OHHA NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS
	Terri Damstra	Biochemist	OHHA NIEHS
	Warren T. Piver	Chemical Engineer	OHHA NIEHS
	Herbert S. Posner	Pharmacologist	OHHA NIEHS

COOPERATING UNITS (if any)
Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This is a long-term project to maintain surveillance on the current state of knowledge of the effects of environmental and other chemical agents on the human body by means of literature searches and such other information as may become available in order to provide advice to Government and other agencies on specific environmental hazards and from time to time to review the current state of knowledge on individual chemical agents. A pilot study is being undertaken to see how far it is feasible to prepare an index of toxicological symptoms and diseases to assist in the differential diagnosis of obscure outbreaks of disease considered likely to be of toxicological origin.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

The object of the project is to assist in providing a service for the assessment of environmental hazards. Thus environmental lead is of continuing interest. The Report of the Committee on Human Health Consequences from Automotive Emissions was edited for publication in Environmental Health Perspectives as a series of articles by members of the Committee. Attention is now being directed towards the relative importance of atmospheric lead and environmental lead dusts as sources of raised blood lead levels. Assistance was given in the preparation of the Report of the Subcommittee on Ethylene Oxide, and help has been given other agencies such as Environmental Protection Agency (EPA) and National Institute for Occupational Safety and Health (NIOSH) in reviewing reports which they have prepared on substances such as atmospheric sulphates and other substances derived from the burning of fossil fuels, and documents as diverse as those dealing with zinc, ethylene dibromide, and acetylene.

The proposed Index of Toxicological Symptoms and Diseases is, if the pilot study shows that the plan is feasible, likely to be a major undertaking.

METHODS EMPLOYED: The standard methods of scientific review and bibliographical research.

MAJOR FINDINGS AND PROPOSED COURSE: This heading scarcely applies as the duties of the Office of Health Hazard Assessment are to provide a continuing service of investigating and assessing environmental hazards which come to the notice of NIEHS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Work of this type is essential if the results of research - both that carried out by the Institute and other research - are to be applied to the needs of the community. Similarly the needs for new research to protect the public from environmental hazards must be identified.

PUBLICATIONS

Martin, A. E. Health Risk Assessment from Epidemiological Data. Prepared for the World Health Organization Committee on Environmental Health Criteria, 1976.

Martin, A. E. Health Aspects of Human Settlements. World Health Organization, Public Health Papers No. 66, Geneva, 1977.

BATTELLE PACIFIC NORTHWEST LABORATORIES - Richland, Washington
(NIH-NIEHS-72-2043)

TITLE: Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $98,500

PROJECT DESCRIPTION

OBJECTIVES: The objectives of these investigations are to determine using advanced radiotracer, spectroscopic, and chromatographic techniques; (1) the rate and extent of uptake of metals from soils by representative grass and broadleafed plants, (2) the translocation of metals in plants, (3) the effect of metals on soil microbiota and soil microbial processes, (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues, and (5) the potential for soil formation of organometal complexes, subsequent uptake of these compounds by plants. Implicit in these objectives are the development of suitable methods for determining the chemical forms of metals in soils, plants, and microbial tissues.

The metals are nickel, cadmium, chromium, and thallium, and the plants are soybeans.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light; 10 hr dark), temperature (27°C light; 20°C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 and 142 days from planting). Plants were separated into cotyledons, roots, stems, leaves, immature bean pods, mature bean pods and mature beans. In all cases the tissues were analyzed for total metal radioisotopes.

In soil biochemical studies, enrichment techniques have been used to select for microorganisms that exhibit a high resistance to added metal concentrations. Procedures employing TLC, column chromatography, electrophoresis, and mass spectrometry are being developed to identify the metabolites of this enrichment process. These same analytical procedures are being used to characterize the chemical form of the metal in plant xylem, leaves, stems, and fruit.

MAJOR FINDINGS AND PROPOSED COURSE: After screening 17 metals for their uptake by barley (Hordeum vulgare) and soybean (Glycine max), and their effect of soil microorganisms, intensive studies have focused on nickel (Ni), cadmium (Cd), chromium (Cr), and thallium (Tl). These four metals were chosen because they exhibited the greatest potential for microbial alteration in soil, and uptake and concentration by soybeans.

A. Microbial Studies

Studies with microbial populations have been toward a better understanding of how these organisms transform the four metals added to soil, and the role these processes have in making metals available for uptake by plants. Specific organisms examined in this regard were fungi, actinomycetes, and aerobic and anaerobic bacteria and spore-forming bacteria. Using the four metals, Cd, Cr, Ni, and Tl, an enrichment program was carried out to isolate organisms capable of growing at high metal concentrations. To expand the enrichment studies, a two-phase procedure was developed to select first for organisms on the basis of carbon sources. Then the isolated metabolic types selected on the basis of carbon source were exposed again to Cd, Cr, Ni, and Tl, but over a broader concentration range.

The form of Cd, Cr, Ni, and Tl was characterized in the exocellular fraction of the mixed fungal and bacterial cultures isolated on the basis of carbon requirements and metal resistance. The experiments in this area were further designed to distinguish between those complexation reactions resulting from reaction with metabolites or normal metabolic processes, and those reactions with the metabolites of metal resistance processes.

As a result of these enrichment studies, it has been demonstrated that growth of organisms with the four metals present, resulted in a change in the chemical form of these metals. These observations have suggested that metabolites formed during growth on different carbon sources, are capable of complexing the metal. These results further suggest that either resistant organisms isolated by these enrichment techniques exhibit an altered metabolism indirectly causing metal modification, or are capable of direct metal modification which may be classified as a detoxification mechanism. Future studies are designed to identify the metal complexes resulting from this transformation.

B. Plant Studies

Chromium was preferentially deposited in the roots, thallium in stem tissue, cadmium in leaf tissue, and nickel initially in the leaves but was remobilized during maturation to seed tissue. 81% of the thallium was associated with the less than 500 molecular weight fraction of stem tissue of soybeans. Whereas 84% of cadmium and 76% of the nickel were associated with molecular weight fractions greater than 10,000 in leaves for Cd and seeds for Ni.

Kinetic studies of nickel uptake have suggested that active accumulation processes using a membrane carrier system employed for nutrient elements is the predominant process for transporting this metal in soybean plants. At

present, investigations are underway to determine if nickel is functioning as a nutrient analog. The results of these studies will shed light on the influence of microbial metabolism on plant availability and ultimate chemical form in the plant. The nickel extracted from the xylem fluid of the kinetic studies had 80% of the total metal associated with molecular weight fractions between 500-10,000. The major portion of this fraction was associated with stable organic compounds consisting of cationic and anionic species of these compounds, several appear to be produced in the root. At present, data are being collected to compare the form of the metal in plants, with the soluble products of microbial metabolism.

C. Biochemical Studies

The actual identification of the chemical nature of these metal complexes extracted from soil fractions, microbial cells, exocellular media and plant components has required the development of new separation procedures. For these reasons, the development of these procedures has been a critical part of this program effort. Techniques and methods of separation which are being applied are ultrafiltration, solvent extraction, electrophoresis, and several types of chromatography. Each method has its application and limitations, and work is continuing to develop methods to provide reproducible results.

PROPOSED COURSE: The objectives of this continuing program are:

A. Microbial Studies

Using the microorganisms isolated by the enrichment programs, studies will be conducted in three major areas. All of these studies are designed to supply additional information on the mechanisms of modification of metals by soil microbial population. These three areas are:

1. Extension of techniques used to identify aerobic bacteria which show resistance to two or more metals, to identify fungal cultures which exhibit this same capability. Metabolites of the alledged modification of the metals by these particular microbial populations will be identified by techniques outlined in the biochemical studies section.

2. Extension of techniques used to identify aerobic bacteria which show resistance to two or more metals, to identify anaerobic metal-resistant organisms and the mechanism by which this resistance is manifested. Again the role of this process in terms of plant availability will be studied by identification of the products of this modification or resistance-to-the-presence of these metals.

3. Validation of *in vitro* studies with isolation of resistant microorganism with soil perfusion column studies. The purpose is to allow evaluation of the importance of the organisms and their metabolites in mediating metal uptake and form in plants.

B. Plant Studies

The three major areas of study are:

1. Analysis of the kinetics of metal uptake, both for each metal itself and for competitive ion effects.

2. Determination of plant availability of metal complexes produced by soil microorganisms in soil perfusion systems and their potential for translocation to edible components of the plant.

3. Chemical characterization of mobile metal form in xylem exudates and deposited form in tissues of soybean plants following uptake of inorganic and organic metal species.

C. Biochemical Studies

Emphasis will continue on detailed characterization of microbial and plant metabolites. Research will be towards improving the selectivity, sensitivity, and efficiency of methods which have been the most promising. In particular, TLC and TLE have been the most effective in identifying and characterizing metal complexes. Continued research is required to improve solvents, buffers, and chromatographic supports and to examine methodological limitations. It cannot be over-emphasized how important this phase of the work is, and how much it can contribute to the theory of mass separation and characterization of materials by chromatography.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. Particular attention should be given to determining the mechanisms for converting inorganic metals to organic complexes and the extent of this conversion. This information should provide a sound basis for estimating the potential hazard arising from the continued use of these materials and may provide the key to remedial measures.

PUBLICATIONS

Drucker, H., Garland, T. R. and Wildung, R. E. The Effects of Metals on Soil Microbial Population and Metabolism: A Ranking System. Presented at the American Society of Agronomy, Soil Science Society of American, Chicago, Illinois, November 1974.

Drucker, H., Wildung, R. E., Garland, T. R. and Fujihara, M. P. Influence of Seventeen Metals on Microbial Population and Metabolism in Soil. Presented at the American Society of Agronomy, Soil Science Society of American, Las Vegas, Nevada, November 1973.

Fujihara, M. P., Garland, T. R., Drucker, H. and Wildung, R. E. Influence of Seventeen Metals on the Relative Distribution and Respiration of Microorganisms in Soil. No. G 37. Presented at the American Society for Microbiology, Washington, D. C., May 1973.

Schneiderman, G. S., Garland, T. R., Drucker, H. and Wildung, R. E. Growth and Chromium Transport Characteristics of Microorganisms Isolated from Chromium Enriched Soil. Presented at the American Society of Agronomy, Soil Science Society of America, Chicago, Illinois, November 1974.

Schneiderman, G. S., Garland, T. R., Wildung, R. E. and Drucker, H. Growth and Thallium Transport of Microorganisms Isolated from Thallium Enriched Soil. Presented at the American Society for Microbiology, Chicago, Illinois, May 1974.

Garland, T. R., Wildung, R. E. and Cataldo, D. A. Accumulation and Distribution of Trace Metals in Tissues of Mature Soybean Plants Grown in a Split-Root Culture System. Presented at the American Society of Agronomy, Soil Science Society of America, Knoxville, Tennessee, August 1975.

Drucker, H., Wildung, R. E. and Garland, T. R. Effects of Chromium on Glucose Metabolism in Soil -- A Potential Biochemical Basis for the Effect of Chromium on Soil Microflora. Presented at the American Society of Agronomy, Soil Science Society of America, Knoxville, Tennessee, August 1975.

Garland, T. R., Wildung, R. E. and Pelroy, R. A. A Rapid Diffusion Method for Physiocochemical Characterization of Metal Ligands in Soils and Sediments. In: Biological Implications of Metals in the Environment, Fifteenth Annual Hanford Life Science Symposium, Richland, Washington, September 29-October 1, 1975.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Quarterly Progress Report and Annual Summary (October 1 to December 31, 1972) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1972.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (February 1 to May 31, 1973) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1973a.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (June 1 to September 30, 1973) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1973b.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (October 1, 1973 to January 31, 1974) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1973c.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (February 1, 1974 to May 31, 1974) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1974a.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (June 1, 1974 to September 30, 1974) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1974b.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (October 1, 1974 to January 31, 1975) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1974c.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (February 1, 1975 to May 31, 1975) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1975a.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (June 1, 1975 to September 30, 1975) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1975b.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (October 1, 1975 to January 31, 1976) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1975c.

Battelle-Northwest. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants, Progress Report (February 1, 1976 to May 31, 1976) to National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 1976a.

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64110
(NIH-NIEHS-72-2090)

TITLE: An Appraisal of Environmental Exposure to Heavy Metals

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer, Office of Health Hazard Assessment

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: $59,277

PROJECT DESCRIPTION

OBJECTIVES: To identify sources and routes of human exposure to heavy metals as a result of the various steps and industrial operations involved in the commercialization, utilization, and disposal of metals and their compounds. To examine and present available information on the toxicology of the metal and its compounds, and to identify analytical methods and their limitations for quantitative measurement of metals in environmental and biological media.

METHODS EMPLOYED: To perform literature reviews and to seek expert opinions from industry in order to compile data on sources of release and exposure to metals and their compounds. Special interest is placed on determination of the physical-chemical properties of the metal that influence how the metal makes contact with man. The information is put together in a report on each metal of specific interest.

MAJOR FINDINGS AND PROPOSED COURSE: Within the past year, a report on zirconium has been produced. These reports have a special significance because they attempt to define the chemical state of the metal in the environment as a result of its release due to intended use or as a contaminant in air and water. The form of the metal and the route of exposure have an important influence on its hazard potential. This type of information distinguishes these reports from the many other reports being produced on metals. Information is being gathered for a number of metals of importance from a toxicological viewpoint. A report on thallium is in press. This is to be followed by a report on indium during the next contract year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals or different forms of metals, or their complexes. The information will determine whether research on the health effects of the compound in question should be undertaken and in which direction it should go. In addition, these documents

have been sent to biomedical researchers who are with many Federal and academic programs, and to program directors in foreign countries. Comments received from these people have indicated that these reports are timely and provide useful information for clinical analysis of patient exposure to metals, and for planning research programs.

PUBLICATIONS

1. Midwest Research Institute: Osmium, An Appraisal of Environmental Exposure Report. Prepared for Contract No. NIH-NIEHS-72-2090. July 10, 1973.

2. Midwest Research Institute: Palladium, An Appraisal of Environmental Exposure. Prepared for Contract No. NIH-NIEHS-72-2090. April 9, 1974.

3. Midwest Research Institute: Silver, An Appraisal of Environmental Exposure. Prepared for Contract No. NIH-NIEHS-72-2090. July 16, 1975.

4. Midwest Research Institute: Zirconium, An Appraisal of Environmental Exposure. Prepared for Contract No. NIH-NIEHS-72-2090. September 9, 1976.

INTERAGENCY PROGRAMS

OFFICE OF ASSOCIATE DIRECTOR FOR INTERAGENCY PROGRAMS
Summary Statement

On behalf of the Director, NIEHS, the Associate Director for Interagency Programs is responsible for the following program areas:

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the *Medical Science and Public Health Cooperative Agreement*, scientists from both countries are conducting joint research on heart disease, cancer, arthritis, influenza and acute respiratory diseases, and health problems associated with environmental pollution. The Director, NIEHS, is U.S. Coordinator for the environmental health activities under the Health Agreement.

1977 was the fifth year of formal collaboration in environmental health research between the US and USSR. The first year was concerned largely with establishing working relationships and agreeing on areas of joint study. Joint research efforts were initiated in the second year of the agreement and involved exchange visits between scientists of both sides. The research results developed during the second and third years of cooperative research were presented by American and Soviet scientists at a symposium in Riga, Latvia, in December 1974. The results of this symposium were published in 1976 in both the United States and the Soviet Union.

Scientific results from cooperative research during 1975 and 1976 were presented at a second joint symposium held in Marineland, Florida, in December 1976. The results of this meeting will be published during 1977 in both the United States and the Soviet Union.

Current collaborative research efforts are divided into four problem areas aimed at developing methods for the quantitative evaluation of the biological effects of environmental chemical agents, for predicting the biological effects of environmental chemical agents, studying the long-term biological effects of environmental chemical agents, and studying the biological effects of physical factors in the environment.

By the end of 1977, over 45 scientific papers will have been published by American and Soviet scientists on the results of environmental health research conducted under this agreement.

The *Agreement on Cooperation in the Field of Environmental Protection* between the US and USSR is aimed at addressing the most important aspects of problems

of the environment, including the study of pollution and its effects on life. A number of agencies participate in this agreement, which is under the general direction of the Administrator, EPA. The Director, NIEHS, serves as HEW representative to the agreement and as Co-Chairman of the working group for one of its areas, the Biological and Genetic Effects of Pollutants. Effort in this area has been focused on the mutagenic potential of environmental contaminants, the toxic effects of heavy metals in the environment, and the potential environmental health hazards associated with the extraction, processing and utilization of oil from shale.

During 1977, program activity under this agreement consisted of the third joint workshop on "Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis" held in San Francisco, and the first joint workshop on "Health Effects of Oil Shale Development," held in Denver, Colorado.

Energy-Related Research

As part of the high priority accorded by the Federal Government to solving the energy problems facing the nation, the Director and staff of NIEHS are involved in a variety of activities to elucidate the potential adverse health impacts of energy technologies.

During the summer and fall of 1974, an OMB/CEQ task force met to identify needed research on the potential adverse health and environmental problems associated with energy use. As part of the report of the Interagency Working Group on Health and Environmental Effects of Energy Use published in November 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. As a result of the work of this task force, NIEHS was assigned the responsibility, and given additional funds, to develop research programs to address a number of important energy-related questions, including the development of physiological indicators to estimate damage to man; the determination of mechanisms of incorporation, metabolism, deposition and turnover of hazardous agents; the determination of the relationship of metabolism and fate and the toxicology of particulates and organic compounds; the determination of the dose-models for extrapolation of cellular and animal data to man; the identification of damage to cells and cell components; and the determination of immediate and long-term effects of critical pollutants on selected ecosystems and organisms in the marine environment.

During the period 1975-1977, NIEHS initiated a number of new projects focused on the problems indicated above. In January 1976, NIEHS co-sponsored, along with NIOSH, EPA, and ERDA, a retreat for about 150 scientists working on energy-related health problems. NIEHS staff continues to participate with staff of other agencies in efforts devoted to the identification and solution of energy-related health problems.

Interagency Coordination

During 1976-1977, a report on Federal Agency Support for Environmental Health Research was completed at the request of the Senate Appropriations Committee. This report summarizes the environmental health research responsibilities,

functions, and coordination efforts of the Department of Health, Education and Welfare, Environmental Protection Agency, Energy Research and Development Administration, National Science Foundation, Department of Agriculture, Department of Commerce, National Aeronautics and Space Administration, Department of Transportation, Department of Housing and Urban Development, Department of the Interior, and Department of Defense. These agencies will spend approximately $528 million for environmental health research and related programs in FY 1978.

INTRAMURAL PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

This is the first full year of operation of this office in the new reorganization of NIEHS. Much time has been devoted to (1) recruiting leaders of new programs (Behavioral Toxicology, Pulmonary Function and Toxicology) or new leaders of existing programs (Biophysics, Pharmacokinetics); (2) developing plans for new programs and expansion of present programs between now and occupancy of the new building; (3) logistics of interim period between now and occupancy of the new building--especially fitting new programs and people into increasingly inadequate facilities; (4) maintaining liaison activities with other groups active in overlapping research areas at NIH and other governmental agencies; (5) developing plans for review of programs by the Board of Scientific Counselors; (6) developing plans for maintaining awareness of intramural programs, contract programs, energy-related research, extramural programs; (7) developing proposals for new NIEHS initiatives in (a) mutagenesis testing and toxicology bioassay programs with NCI, and (b) for support of centers in marine and aquatic biomedicine and further development of our intramural programs in this area here, in Maine and in Florida; and (8) development of plans to add more support to the Scientific Director's Office.

Accomplishments include the recruitment of Dr. Clifford L. Mitchell to head the Behavioral Toxicology Group and the subsequent staffing of that group with interdisciplinary expertise; the recruitment of Dr. Paul Nettesheim to be Chief of the new Laboratory of Pulmonary Function and Toxicology and the planning for the staffing of that unit over the next 2-3 years; the recruitment of Dr. Colin Chignell to be Chief of the Laboratory of Environmental Biophysics and the planning for establishment of a new program applying sophisticated biophysical techniques to studies of chemical effects, in addition to the present programs in that branch.

The Board of Scientific Counselors met twice during the year to review Intramural programs. Two of the larger laboratories presented their programs to the Counselors--Laboratory of Environmental Toxicology and Laboratory of Environmental Mutagenesis. The Counselors will be reviewing each of the intramural laboratories at least once every 3 years and sometimes more often. The Laboratory of Environmental Toxicology has now been reviewed twice since its establishment in 1972 (1974 & 1977). In the next year, the Laboratories of Pharmacology and Biometry will be reviewed. The practice of supplementing the Board of Scientific Counselors with ad hoc reviewers in research areas not represented by Board members will be continued as this results in in-depth reviews of all program areas most efficiently.

An NIEHS Science Seminar was held at the Carolina Inn, UNC, Chapel Hill, N.C., June 2-3, 1977. At this seminar, NIEHS scientists presented as complete a

picture as they were able of the intramural research program--in the form of lectures and posters. Invitees included all other governmental agencies with research interests similar to, overlapping with, or tangential to ours; e.g., NIOSH-OSHA, EPA, NCTR, all other NIH's (and especially NCI, NICHHD, NIMH, NHLBI, NIAMDD), FDA, as well as some representatives of the press, public interest groups, and non-governmental research groups (e.g., our University Centers, local universities, and research institutes) to the limit of our attendance capacity (200). We hope that by such efforts, all who wish/need to know of our research program will have a chance to personally meet, talk with, and exchange ideas with our scientists who are actually doing this work. We propose to have such sessions in the future though the frequency is not yet set.

An intensive look at the recommendations made in the second task force report on Research Planning in Environmental Health Sciences was organized by the Scientific Director, the Director for the Research Resources Division, and the Director of Extramural Programs. An attempt was made to consider each one of the recommendations in this report in terms of what NIEHS as a whole was now doing, wished to do but needed more resources for, would do but in somewhat different way or at a different level of priority, or would not be able or felt it unwise to do at present or in the near future. A Subcommittee of the National Advisory Council will be working with intramural and other NIEHS Scientists and present a written report on NIEHS and the Task Force II document sometime in fall/winter 1977.

A process was begun to study how decisions are made within the Intramural and Research Resources programs and how to more effectively make major policy decisions and interact with other parts of the NIEHS. This is seen as a continuing process and one which, if even partly successful, will allow greater input to the decision-making processes by all scientists/program leaders at NIEHS and lead to more satisfying and rapid methods for settling differences and establishing effective interactions up, down, and across various administrative lines at NIEHS.

The Scientific Director meets regularly with other Scientific Directors at NIH in Bethesda to discuss various inter-institute matters such as appointment and promotion plans, policies and reviews, EEO and affirmative action plans, rules for recombinant DNA research, Boards of Scientific Counselors' reviews of intramural research, travel plans and policies, contracts and monitoring, employment ceilings and grades, central research and research support facilities (libraries, computers, clinical pathology), clinical research activities, post-doctoral and visiting scientist plans, policies and review, personnel management and supervisory training efforts. These meetings are at least twice a month and assure that institutes do not develop procedures and policies at wide variance or threatening to the concept and practices of NIH as a whole. There is also a sharing of information about the research programs at other institutes and this helps in collaboration, avoidance of unnecessary duplication, etc.

The Scientific Director's Office was called upon to present descriptions of the intramural research program to a variety of visitors and to outside groups on a number of occasions--among these (1) a delegation of scientists from the

People's Republic of China, (2) visitors from Yugoslavia seeking possible areas of mutual research interest, (3) visitors from Egypt meeting with Dr. Rall and Dr. Moore concerning the new U.S.-Egypt Environmental Health Sciences Agreement, (4) NIEHS Center Directors (extramural programs), and (5) NIEHS National Advisory Council.

A number of lectures and seminars were given by the Scientific Director--some on his own personal research (e.g., at University of California, Davis), also lectures on pharmacology-toxicology to various students (e.g., School of Nursing, UNC, on health effects of environmental pollutants, perinatal pharmacology and toxicology), and papers on the toxicology of environmental pollutants at various scientific meetings (e.g., carcinogenic effects of chemicals at U.S.-Japan Cooperative Cancer Research Conference).

A number of other professional activities included activity as Chairman of the Drug Metabolism Division of the American Society of Pharmacology and Experimental Therapeutics; Co-Chairman, Gordon Conference on Drug Metabolism; Basic Pharmacology Advisory Committee, Pharmaceutical Manufacturers Association Foundation, Inc.; and editorial boards of Cancer Research, Chemico-Biological Interactions, Xenobiotica, and the Journal of Toxicology and Environmental Health. In the present fiscal year--October 1976 to September 1977--the Scientific Director published 5 articles on his personal research (e.g., Xenobiotic metabolisms in skin, lung, marine species), 3 general overviews or selected viewpoints on science or science issues, and one review in-depth on drug metabolism in birds (a book).

BIOMETRY BRANCH

BIOMETRY BRANCH
Summary Statement

The primary mission of the Biometry Branch is to conduct applied research in the areas of biomathematics, epidemiology, and risk assessment. The Branch also provides statistical, mathematical, data processing and computer engineering support to the Institute.

Biomathematics, which is essentially a collaborative effort involving a variety of scientific disciplines, focuses on mathematical modelling of biological processes. At the present time the major areas of interest are mathematical population genetics and pharmacokinetic modelling. The Branch's epidemiological research is concerned with the evaluation of the impact on human health of exposure to environmental levels of various potentially hazardous compounds. Hazard assessments are made directly through actual field studies as well as indirectly by the analysis of pre-established data bases. In addition methodological research directed toward improved planning and evaluation of epidemiological studies is also conducted. Risk assessment activities have emphasized the improvement of the use of data generated from animal screening studies to estimate long-term human risk from exposure to ambient levels of chemical carcinogens. Research efforts range from the development of sophisticated mathematical models for extrapolation to the investigation on an applied, semi-quantitative basis of species differences in response to carcinogenic agents.

Statistical support given to Institute intramural scientists spans the spectrum of consulting activities from design of pilot studies to analysis of data generated at the conclusion of large-scale, long-term experiments. Computer support covers such diverse activities as the development of information retrieval and inventory maintenance systems, the simulation of complex biological models designed to clarify mechanistic processes, and the provision of computer engineering support for real time and minicomputer data acquisition and controls systems.

Biomathematics

Research is underway in two areas of mathematical biology. Both programs involve collaboration with experimental scientists in other branches.

Joint research with the Laboratory of Environmental Mutagenesis is being conducted on problems in population genetics theory. The long range objective of this work is to develop a better understanding of environmental effects on gene frequency distributions. A model for spatial dispersion of neutral alleles was used in conjunction with

drosophila data to indicate the improbability of the same neutral allele appearing at widely separated localities. Some mathematical results needed to study the effect on natural selection of autocorrelated changes in the environment were obtained. A probabilistic relation between the age of a neutral allele and its frequency was derived.

In collaboration with the Laboratory of Pharmacology, research efforts in pharmacokinetics were concentrated in two areas: 1) whole body distribution and modeling studies, 2) uptake storage, metabolism, and release of pollutants and model compounds by isolated perfused organs, with present emphasis on liver and lung. The kinetics of chemical disposition in whole body and isolated organs are being determined in order to construct mathematical models of these processes which can be used: (a) to better define and understand rate-limiting steps in the process, (b) to extrapolate distribution profiles of chemicals from one tissue to another, one species to another, and to man, and (c) to predict tissue storage of pollutants from knowledge of routes, dose, and numbers of exposures. The disposition of five polychlorinated biphenyls (PCB) isomers was studied in the rat and mouse. Pharmacokinetic models have been developed for the rat and these can be scaled to the mouse. Disposition data is being obtained for the same compound in the dog and monkey so that species-to-species extrapolation of disposition profiles can be made. Several of these compounds have extremely long half-lives of storage in skin and fat. The pharmacokinetic models will be used to predict accumulation from long term low-level exposure. The disposition of a polybrominated biphenyl, 2,4,5,2',4',5'-hexabromobiphenyl has also been investigated. The rat is essentially incapable of metabolizing this compound and hence its half-life is greater than the life span of the animal. Pharmacokinetic models have also been developed for this compound and prediction of accumulation from chronic dosing is possible. The correlation of pharmacokinetic parameters with toxicological parameters is under investigation.

Risk Assessment

Use of data from animal experiments to estimate the human risk from long-term exposure to very low doses of environmental carcinogens poses a number of biological, pharmacological, and statistical problems. One of the statistical problems is to extrapolate the animal dose-response relations from the high dose range where animal test data are available to low doses which humans might encounter. Different techniques which would seem to fit the test data equally well can lead to low dose risk estimates that differ by several orders of magnitude. The purpose of this project is to evaluate existing methods and to develop new statistical methods for risk estimation and animal test design, reflecting current understanding of carcinogenic mechanisms.

Methods for low-dose extrapolation based on the Armitage-Doll multistage carcinogenesis model were incorporated into computer programs and used to estimate the cancer risk associated with a number of different chemicals, including some trace impurities in drinking water. Computer programs were developed to simulate patterns of dose-response data such

as are obtained in animal carcinogenesis experiments. These programs are being used to investigate the statistical uncertainty in low-dose extrapolations from animal experiments and to make a preliminary exploration of some considerations in the statistical design of such experiments. Statistical methodology was developed for some hypothesis tests associated with low-dose extrapolations. The computer programs used in this work have been made available to other scientists at NIH.

Mathematical models to predict the effect of dose on cancer latency period were investigated. This work was motivated by data which had been cited in the literature as implying a physical increase in tumor growth time with decreasing dose. It was shown that the observed increase in average latency period could also be explained as a simple mathematical consequence of a decrease in incidence with decreasing dose; no physical increase in tumor development time need be postulated to explain the data. The average latency period and the minimum latency period in a small group of animals were both shown to be poor indicators of the risk of developing a tumor within a normal animal lifespan.

Epidemiology

While the focus of the epidemiology program has remained unaltered, i.e., the identification of potential health hazards in the general environment, the scope of the program has been broadened considerably by the initiation of field studies for direct hazard assessment. One of the first issues that is being addressed by this mechanism is the evaluation of the possible excess risk incurred by breast fed infants as a result of their exposure to elevated levels of PCB's (polychlorinated biphenyls) in mother's milk. A prospective study has been launched to identify and assess the health status of a cohort of children at birth and then to periodically reexamine them over the first six months of life. Concurrently, breast milk and formula samples, as well as cord blood and placenta, will be tested for evidence of contamination. Although the study is initially restricted to the state of North Carolina, it is hoped that a flexible field methodology will be developed, allowing the problem to be evaluated in other locals while maintaining tight data comparability. Another environmental hypothesis that is being explored in an actual field setting is concerned with the potential effects of lead exposure on the cardiovascular system in children who have measurable body burdens of lead but are not clinically intoxicated.

In addition statistical methodology problems relevant to epidemiological research continue to be investigated. These statistical investigations are directed at such diverse issues as the usefulness of ecological associations as a means of evaluating environmental risk, the estimation of prevalence rates through screening tests, and the assessment of synergism (or antagonism) using epidemiologically-based data.

Statistical Consulting

While much of the Biometry Branch's focus is on its own research efforts, it also provides a statistical consulting service for the intramural

research program. Statistical consulting is quite comprehensive in its scope, encompassing both experimental design and data analysis.

The design of experiments covers all types of investigations ranging from small-scale pilot studies to experiments concerned with the effects of lifetime exposures to potentially hazardous chemicals. Besides the usual emphasis on the maximization of design efficiency, special attention is given to the particular, practical constraints which confront the individual investigator. As a result, the assessment of study feasibility is one of the most useful consulting services provided in this area.

Data analysis involves the use of all the traditional parametric and non-parametric statistical procedures. For large data sets, or studies requiring the use of complex statistical methods, data analysis activities are closely coordinated with the Branch's computing work group. In addition to these services there is also a very active applied research effort directed toward the development of new methodology to meet some of the highly specialized analytical needs of the intramural program.

In order to partially relieve the increased consulting workload created by the expansion of the intramural research program, additional emphasis has been placed on the continuing development of self-help systems for routine data analysis. These systems have been made readily accessible to all Institute scientists and technicians. Moreover, a basic course on statistical design and analysis is now being offered to members of the intramural program, emphasizing the role which statistics should play in their various research efforts.

Computing

Data processing efforts within the Biometry Branch fall into two distinct areas - those directed principally to tasks within the branch, and those dealing primarily with providing data processing and computer engineering services to the Institute at large.

Numerous projects in the latter category have received support. Those of an administrative nature include a computerized warehouse inventory management system, a periodically updated listing of all radioisotopes held at NIEHS, a budget reporting system listing financial transactions and billings with summaries for each branch, and an animal ordering and inventory system.

In support of intramural research, major efforts have been conducted in the fields of protein receptor analysis for the Developmental Toxicology section, assessment of synergism for the Inhalation Toxicology section, and gene frequency deviation detection with Monte Carlo simulation of related phenomena for the Population Genetics section. The Pharmacokinetics section continues to expand its use of computing facilities. In addition to this section's on-going analyses of pharmacokinetic data, numerical methods requiring extensive computational power are under development. These techniques will provide the mathematical modelling and computer simulation facilities needed for inter-species extrapolation of low-level, long term pharmacokinetic profiles.

A capability for providing computer engineering support to the laboratories of the Institute is presently being developed within the Biometry Branch. Solutions will be sought to engineering problems related to all aspects of computer hardware and instrumentation programming. The tasks of this effort to date have involved the specification of minicomputers, peripherals, and operating system software; the design of interfaces between these minicomputers and laboratory instruments; and the development of software for control of experiments, data acquisition, and data transmission. The following major tasks are currently underway: (1) For the Laboratory of Environmental Mutagenesis, computer programs are being developed for the PDP-12/Zeiss system for absorption measurements of stained sperm cells and partially-automated measurement of mutation frequency in sperm and red blood cells. As this research project progresses, hardware and software will be recommended for the fully-automated measurement of mutation frequency. (2) A network of minicomputers has been recommended for the Behavioral Toxicology Program. Interfaces between these computers and the behavorial apparati are being designed and built. (3) For the Laboratory of Environmental Mutagenesis, a small minicomputer system is being developed for the automatic acquisition and storage of data from a Gilford spectrophotometer. Also an X-Y digitizer will be interfaced to this computer for measurement of DNA molecules.

Within the Biometry Branch, substantial efforts have been directed toward improving the general data processing capability, particularly as it affects the computing needs of those providing statistical data analysis in support of the intramural research program. The Institute's PDP 11/40 computer has been significantly expanded with the addition of various peripherals and the implementation of a multi-programming operating system. This computer is heavily used both to provide high-speed telecommunications to the NIH IBM/370 system and to accomplish a great variety of local computing activities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 40002-07 BB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. K. Haseman	Mathematical Statistician	BB NIEHS
Other:	B. C. Gladen	Staff Fellow	BB NIEHS
	F. O. Lin	Visiting Fellow	BB NIEHS
	L. L. Kupper	Guest Worker	UNC

COOPERATING UNITS (if any)

Department of Biostatistics, University of North Carolina

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's consulting activities. These efforts include the development of new statistical tests and the modification of existing procedures to meet specific Institute needs.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical methodology ranging from Monte-Carlo simulation techniques to the theoretical modification of existing test procedures has been utilized to produce analytical techniques applicable to problems arising in various intramural laboratory studies.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Neither the Poisson nor binomial models were found to explain adequately the distribution of fetal death in many sets of animal data. A beta-binomial model was found to provide a markedly improved fit, but an alternative correlated binomial model was derived that was superior in many cases. The use of jackknife techniques for parameter estimation and hypothesis testing in this situation was also investigated. (2) Monte Carlo techniques were employed to investigate the significance level and power of a number of nonparametric multiple comparison tests. Certain of these procedures appeared to be unduly conservative when the number of groups being compared was large. (3) For pairwise comparisons involving incidence data, an arc-sine approximation is frequently used to determine required sample sizes for achieving a given level of power. This approximation was found to underestimate sample sizes in some instances by as much as 20-25%, and tables of exact sample sizes based on Fisher's exact test for 2x2 table were calculated for a number of different underlying incidence rules.

The proposed course is to continue research effects on statistical methodology problems that arise as a result of the intramural consulting activities in the Biometry Branch.

SIGNIFICANCE TO BIOMETRICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of improved statistical methodology as the need arises is essential if the quality of statistical consulting provided to the intramural program is to be maintained at a high level.

PUBLICATIONS

Haseman, J.K. and Soares, E.R.: The distribution of fetal death in control mice and its implications on statistical tests for dominant lethal effects. Mutation Research 41: 277-288, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 41001-03 BB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Risk Assessment for Environmental Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Hoel	Chief	BB NIEHS
	H. A. Guess	Research Mathematician	BB NIEHS
Other:	K. S. Crump	Consultant	LA Tech. Unv.
	C. H. Langley	Section Head	LEM NIEHS
	R. Peto	Consultant	Unv. of Oxford

COOPERATING UNITS (if any)

Laboratory of Environmental Mutagenesis

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long term risk from chemical carcinogens at very low dose levels. Existing techniques are evaluated and new techniques are studied, with particular emphasis on those which attempt to relate mechanistically to the carcinogenic process.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Various possible models of carcinogenesis were analyzed with respect to low dose kinetics. Considerations of mechanisms such as hit models and multistage models were compared to so-called log normal response models. The importance of background incidence and induction time were evaluated. These considerations led to the investigation of "best" risk estimates and optimal experimental designs.

MAJOR FINDINGS AND PROPOSED COURSE: Statistical methodology based on the Armitage-Doll multistage model has been developed and used to analyze dose-response data from animal experiments with a number of chemical compounds and from computer simulations of such experiments. This work has produced the following conclusions applicable to carcinogens that are directly on cellular DNA: (1) If the dose-response trend is linear in the range where test data are available, then the total statistical uncertainty on the risk at low dose levels will be small, provided that the sample sizes are fairly large (e.g., 200 animals per dose). (2) If the dose-response trend in the range where test data are available is nonlinear, then the upper confidence limit on the difference between the risk at a given dose and the background risk will often be as high as one in ten thousand at dose levels where the best estimate of this increased risk is around one in one million. (3) At low dose rates the upper confidence limits on increased risk are approximately linear functions of dose, even when the best estimate curve of increased risk versus dose is nonlinear.

Mathematical models for predicting the effect of dose on cancer latency period were investigated; it was found that both the average latency period and the minimum latency period in a small group of animals were poor indicators of the risk of developing a tumor within an animal lifetime.

Future work will involve (1) developing the statistical methodology for using time-to-tumor data from animal experiments to improve the accuracy of low-dose risk extrapolations and to account for effects of competing risks, and (2) evaluating species-to-species variability in carcinogenicity data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project potentially will have an impact upon human risk assessment of environmental carcinogens. This in turn should affect the regulatory process. Further, the techniques developed in this project may be applicable to toxicological effects other than carcinogenesis.

PUBLICATIONS

Crump, K. S., Hoel, D. G., Langley, C. H., and Peto, R.: Fundamental carcinogenic processes and their implications for low dose risk assessment. Cancer Res. 36: 2973-2979, 1976.

Guess, H. A. and Crump, K. S.: Low-dose extrapolation of data from animal carcinogenicity tests - analysis of a new statistical technique. Mathematical Biosciences. 32, 15-36, 1976.

Hoel, D. G.: Statistical extrapolation methods for estimating risks from animal data. NYAS Conference on Occupational Carcinogenesis. Proc. Annals of the New York Academy of Sciences. 271, 418-420, 1976.

Guess, H. A. and Crump, K. S.: Best estimate low dose extrapolation of carcinogenicity data. Environ. Health Persp. (in press).

Guess, H. A. and Crump, K. S.: Can we use animal data to estimate safe doses for chemical carcinogens? In Whittemore, A. S. (Ed.): Environmental Health: Quantitative Methods. Philadelphia, SIAM (in press).

Guess, H. A. and Hoel, D. G.: The effect of dose on cancer latency period. J. Tox. and Environ. Health. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 42002-05 BB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Disposition, Pharmacokinetic Models, and Species-to-Species Extrapolation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Anderson	Mathematician	BB NIEHS
Others:	T. E. Eling	Research Chemist	PB NIEHS
	H. B. Matthews	Research Chemist	PB NIEHS
	D. B. Tuey	Guest Worker	BB NIEHS
	R. L. Dedrick	Section Chief	BEIB DRS
	R. J. Lutz	Chemical Engineer	BEIB DRS

COOPERATING UNITS (if any)

Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH
Biometry Branch

SECTION
Pharmacokinetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to understand the mechanisms which determine the disposition of environmental chemicals in various animal species and to develop pharmacokinetic models for these chemicals. (1) After developing pharmacokinetic models in several species, it may be possible to extrapolate disposition of chemicals to other species, and, ultimately, to man. The prediction of the accumulation in man after low-level chronic exposure could then be done. Pharmacokinetic information (models) can be very useful in the design and interpretation of toxicity studies. The topics of present interest are: (a) disposition of several polychlorinated biphenyls in the rat, mouse, dog, and monkey. (b) development of pharmacokinetic models for the polychlorinated biphenyls in these species.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The approach used by R. L. Dedrick and D. L. Zaharko in the development of pharmacokinetic models for several drugs is being employed to develop similar type models for environmental pollutants.

MAJOR FINDINGS AND PROPOSED COURSE: The major findings are: (1) Determination of basic disposition parameters such as metabolic rates, urinary and biliary excretion rates, protein binding affinities, partitioning constants between blood and tissues, etc. is necessary to understand the disposition of a chemical. These parameters can be used to construct physiological pharmacokinetic models. Pharmacokinetic models have been developed for 4-chloro-, 4,4'-dichloro-, 3,5,3',5'-tetrachloro-, 2,4,5,2'5'-pentachloro- and 2,4,5,2',4',5'-hexachlorobiphenyl, in the rat. These models can predict the disposition up to 42 days after a single i.v. dose. The biochemical and physiological parameters used in this model were determined from studies in bile-cannulated animals, isolated perfused liver studies, and disposition studies following a single i.v. dose. The adipose to blood partitioning coefficient for the hexachlorobiphenyl that we observed in the rat was similar to that reported for humans. (2) A scaled version of the model constructed for the polychlorinated biphenyls in the rat gives at least a good qualitative description of the disposition of PCB in the mouse. The disposition of 2,4,5,2',4',5'-hexabromobiphenyl has been examined in the rat. More than 90% of this compound remained in the animal 42 days after a single i.v. dose of 0.6 mg/kg. A pharmacokinetic model has been constructed for this compound in the rat. The model's prediction of the disposition of the hexabromobiphenyl after multiple oral doses was very accurate.

The proposed course is: (1) Disposition studies of these PCBs will be done in rat and mouse after multiple doses. The ability of the models to predict disposition in these species after multiple doses will be tested. (2) Data for the disposition of these PCBs in monkey and dog will be obtained through a contract. This data will be used to construct models in these species. (3) Values for the metabolic kinetic parameters are the most difficult to obtain. Additional experiments are being planned which will enable us to determine better estimates for these parameters. (4) Disposition of the major metabolites of 4-chloro and 2,4,5,2',5'-pentachlorobiphenyl will be studied. These studies will enable us to obtain a better understanding of the bilary and urinary excretion of the metabolites of polychlorinated biphenyls. (5) There is an initial rapid accumulation of the polychlorinated biphenyl in the liver after an i.v. dose. We will examine the mechanism of this accumulation. (6) From a comparison of the parameters used in the models for the various species, we want to extrapolate to values of these parameters for man. Then a pharmacokinetic model for man can be constructed. Predictions of accumulation in man after low-level exposure will be made. (7) Attempts will be made to obtain disposition data for xenobiotics in man. We are currently involved in two projects

which will hopefully give us some information, these being studies on the pharamcokinetics and toxicity of Kepone in humans and similar studies with the polybrominated biphenyls.

PUBLICATIONS

Lutz, R.J., Dedrick, R.L., Matthews, H.B., Eling, T.E., and Anderson, M.W.: A preliminary model for several polychlorinated biphenyls in the rat. Drug Metabolism and Disposition. (in press).

Anderson, M.W., Eling, T.E., Lutz, R.J., Dedrick, R.L., and Matthews, H.B.: The construction of a pharmacokinetic model for the disposition of polychlorinated biphenyls in the rat. Fourth Deer Lodge Conference on Clinical Pharmacology. Journal of Clinical Pharmacology. (in press)

Tuey, D.B., and Matthews, H.B., The effect of chlorine position on 3,3',5,5'-tetrachlorobyphenyl pharmacokinetics in the rat. Drug Metabolism and Disposition. (submitted)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43001-05 BB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Epidemiological Investigations of Potential Human Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. D. Hogan	Mathematical Statistician	BB NIEHS
	W. J. Rogan	Staff Associate	BB NIEHS
Other:	P. Y. Chi	Visiting Fellow	BB NIEHS
	B. C. Gladen	Staff Fellow	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of epidemiological investigations. In support of this effort, attention is also given to the development of statistical methodology applicable to environmental epidemiology problems. As part of this project a study of the association between the chloroform levels in finished drinking water supplies and site-specific cancer mortality has been conducted, and an investigation of the cardiovascular effects of juvenile, subclinical lead intoxication has been initiated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) The study of the potential relationship between drinking water contamination and cancer mortality has been completed. Of the various sites considered, the rectum-intestine and bladder displayed the strongest association with chloroform levels. However the results were shown to be highly dependent on both the weighting scheme and the specific explanatory variables included in the analysis. (2) The analysis of data on blood lead, hematocrit, height, weight, sex, age, and blood pressure, abstracted under contract from the medical charts of children participating in a county-based lead screening program will be completed; and the possible cardiovascular effects of subclinical lead intoxication will be evaluated. (3) The review, modification, and development of statistical methodology relevant to environmental health issues, such as the assessment of synergism, the estimation of incidence rates from survey data, and the evaluation of the effects of time trends on age-groupings in mortality analyses will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: (1) The investigation of chloroform and cancer mortality served the dual purpose of identifying specific sites that may need to be evaluated by a direct study as well as indicating the limitations of the ecological approach to environmental health issues. (2) Lead is the most widely known and important environmental chemical in pediatrics, and research in children with body burdens below acute intoxication has received little attention. Blood pressure is an extremely prevalent cardiovascular abnormality, and is an important predisposing factor to myocardial infarction, congestive heart failure, and stroke. The discovery of a causal and treatable relationship between the two would be highly significant.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43002-01 BB

PERIOD COVERED
August 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Environmental Contaminants of Infants Foodstuffs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Rogan	Staff Associate	BB NIEHS
Other:	B. C. Gladen	Staff Fellow	BB NIEHS
	J. A. Moore	Acting Associate Director Research Resources Program	OD NIEHS
	J. D. McKinney	Supervisory Chemist	EBCB NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch; Office of the Director

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [X] (b) HUMAN TISSUES [ ] (c) NEITHER

[X] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Low level organochlorine contamination of breast milk is a well documented phenomenon, but the health effects on infants fed such milk are unstudied. This project will establish a cohort of breast fed and formula fed infants; the children will be evaluated for specific outcomes thought to be related to organochlorine exposure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is a prospective, or follow-up study. Field nurses are hired, trained in protocol administration, and then work at selected hospitals. Subjects are enrolled, informed consent is obtained, and a questionnaire administered to each mother at about the time of delivery. Samples of milk, formula, colostrum, placenta, and maternal blood are collected. The children are examined, and follow up appointments made. Several examinations are performed over the first 6 months of the child's life.

MAJOR FINDINGS AND PROPOSED COURSE: No data analysis is yet complete. Data collection in North Carolina will continue. Selected sites outside the state will be enrolled to allow wider range of dose maintaining tight data comparability.

Our objectives in continuing this study are (1) to determine the prevalence of environmental contamination of breast milk in North Carolina; in particular, to determine the levels of PCB and total organic chlorine; (2) to establish the relationship between milk levels, maternal blood levels, cord blood levels, placental levels, and colostrum at birth, and examine the trends in milk concentration over time; (3) to investigate the relationship between PCB and TOC levels in the neonate and a number of specific outcomes; (4) to follow breast fed and non breast fed infants over a period of six months and look for differences in incidence of a number of specific outcomes; (5) to generate other hypotheses about toxic effects of chronic low dose PCB exposure in children, and (6) to establish a cohort of children for follow up studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health effects of low dose environmental pollutants are completely obscure, and this project should allow identification and quantification of those that occur short term in this high risk group. The methodology for studying such phenomenon is also of interest, and the development of a field efficient method for study of low level pollutants in humans is important.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-02 BB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Guess	Research Mathematician	BB NIEHS
Other:	J. H. Gillespie	Consultant	Unv. of Penn.
	C. H. Langley	Section Head	LEM NIEHS
	K. S. Crump	Consultant	La. Tech. Univ.

COOPERATING UNITS (if any)

Laboratory of Environmental Mutagenesis

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to develop a better understanding of environmental effects on gene frequency distributions. The work is conducted in collaboration with scientists in the Laboratory of Environmental Mutagenesis. Current research topics are: (1) the relation between the frequency of a neutral allele and its age; (2) effects of autocorrelated random environments on the progress of natural selection; (3) an assessment of the accuracy of existing models for predicting the transient behavior of recessive lethal alleles; (4) theoretical developments in stochastic differential equations applicable to problems in population genetics.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: To work jointly with scientists in LEM on problems in population genetics theory. This work, along with experimental population genetics studies conducted by LEM, is aimed at developing a better understanding of environmental effects on gene frequency distributions.

MAJOR FINDINGS AND PROPOSED COURSE: A new diffusion approximation was developed to predict the effect of autocorrelated random changes in environmental conditions on the way natural selection operates on gene frequency distributions. Some work was started to assess the accuracy of existing models for predicting the transient behavior of recessive lethal genes in large populations. Basic theoretical work was started to extend current results in stochastic differential equations applicable to population genetics. Future work will continue in these areas. Increased effort is planned in medical genetics and in topics in stochastic processes needed to study random environmental effects on gene frequency distributions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of environmental effects on gene frequency distributions contributes to a knowledge of how genetic variability should be taken into account in predicting long-term effects on environmental changes in public health.

PUBLICATIONS

Crump, K. S. and Gillespie, J. H.: The dispersion of a neutral allele considered as a branching process. J. Appl. Prob. 13, 208-218, 1976.

Guess, H. A. and Gillespie, J. H.: Diffusion approximations to linear stochastic difference equations with stationary coefficients. J. Appl. Prob. (in press).

Gillespie, J. H. and Guess, H. A.: The effect of autocorrelation on the progress of natural selection in random environments. American Naturalist. (in press).

Watterson, G. A. and Guess, H. A.: Is the most frequent allele the oldest. Theoretical Population Biology. (in press).

American Health Foundation - New York, New York
(N01 ES 6-2130-01)

TITLE: Species-to-Species Carcinogenesis Extrapolation

CONTRACTOR'S PROJECT DIRECTOR: G. M. Williams, M.D.

PROJECT OFFICER (NIEHS): M. D. Hogan, Ph.D., Mathematical Statistician, Biometry Branch

DATE CONTRACT INITIATED: June 15, 1976

CURRENT ANNUAL LEVEL: $53,542

PROJECT DESCRIPTION

OBJECTIVES: To create a data summary from published studies in the field of carcinogenesis and to utilize this data summary to investigate various aspects of the quantification of species differences following exposure to specified carcinogenic agents. These differences will in turn be incorporated into the species-to-species extrapolation process whenever feasible.

METHODS EMPLOYED: All relevant pharmacological and epidemiological literature on those agents which have been found to be carcinogenic in man and at least one animal species is being reviewed and abstracted.

PROPOSED COURSE: Once the literature review is completed, the abstracted data will be used to evaluate various questions that are pertinent to the issue of species-to-species extrapolation, such as the establishment of dosage equivalencies, estimation of a metabolic activation/detoxification factor, etc.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the absence of relevant human data, decisions regarding acceptable levels of various environmental agents are increasingly being based on information derived from animal experimentation. One of the major obstacles to the development of realistic and highly explicit animal models that will accurately predict human response is the problem of species-to-species extrapolation. If we are to significantly reduce our reaction time in responding to environmentally based threats to human health through the vehicle of the animal test system, then it is essential that we successfully confront the quantitative problems involved in species-to-species extrapolation.

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

LABORATORY OF ENVIRONMENTAL BIOPHYSICS
Summary Statement

The Laboratory of Environmental Biophysics is primarily concerned with the biological effects of physical factors present in our environment. At the present time, the efforts of the Branch are focussed primarily in two main areas, namely non-ionizing radiation (microwaves) and noise (including both auditory and nonauditory effects).

Non-ionizing radiation

The objectives of the non-ionizing radiation research program are as follows: 1) to develop microwave exposure systems for bioeffects research, 2) to develop and test techniques for measuring microwave energy absorption, 3) to determine the effect of microwaves on isolated nerve preparations, 4) to determine how 2450 MHz microwave radiation interacts with biological systems at all levels, 5) to study the effect of long-term exposure of experimental animals to 915 MHz and 2450 MHz microwave radiation on their CNS and behavior, 6) to ascertain the effect of 60 Hz fields from high voltage transmission lines on the CNS of mammals.

During the past year a waveguide system for exposing isolated nerves to microwave radiation has been fabricated and tested. In addition, a 2450 MHz absorber lined horn system has been modified to permit exposure of pregnant mice. Equipment has also been built to measure the colonic temperature of mice during microwave exposure. Studies on the biological effects of 2450 MHz radiation on a variety of in vitro systems has failed to reveal any significant changes in the irradiated systems other than those that could be directly attributable to heating. However, a small decrease in fertility was observed when male quail that had been exposed in ovo to microwaves were mated with either control or exposed females.

One of the most important areas in non-ionizing radiation research is the effect of prolonged low level exposure to microwaves. The long-term effects of the exposure of rats to 915 MHz and 2450 MHz is currently the subject of investigation by two contractors. Preliminary results suggest that exposure of pregnant rats to 915 MHz radiation does not alter the relative numbers of viable offspring and stillborns. Studies on the long-term effects of microwave exposure on the CNS and behavior of rats are still ongoing. The effect of long-term exposure of rats to 60 Hz fields is the subject of a third contract.

Noise

Much of the effort in the noise program is directed towards a better understanding of both the physiology of the inner ear and the basic transduction

mechanism whereby sound is converted into electrical nerve impulses. Such basic studies are necessary before the effects of auditory and chemical insults on the hearing process can be understood.

Areas under current investigation include (1) the role of electrolyte composition and flux on the functioning of the guinea pig cochlea, (2) the development of an optical fiber motion detector for auditory system measurements, (3) the development of electrophysiological techniques to recover speech from the cochleas of live, anesthetized guinea pigs, (4) the effect of noise and ototoxic agents, e.g., methyl mercury on the electrolyte metabolism of the cochlea, (5) the effect of ototoxic insult on the coding of complex signals in the auditory system and (6) the development of an impact noise generator for hearing loss studies.

In order to understand the basic mechanisms of electrolyte transport in the guinea pig cochlea, the flux of K^+, Na^+ and Cl^- from the perilymph to endolymph has been studied using isotopic techniques. The endolymph actively takes up K^+ and extrudes Na^+. Ouabain and anoxia both suppress this active transport. While chloride normally diffuses passively from perilymph to endolymph, anoxia decreases the uptake of Cl^- by the endolymph. The effect of exposure to intense noise on the cochlea electrolyte levels is currently under investigation. Methyl mercury, a known ototoxic agent, causes suppression of the cochlea and microphonic potential while the endolymphatic potential remains unchanged.

Work has continued on the development of an optical fiber motion detector for measurement of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL). Such a system would permit reliable measurement of the motion of the basilar membrane. Experiments with speech recovered from the inner ears of anesthetized guinea pigs indicate that signals imparted by normal acoustic methods or via direct activation with piezoelectric transducers are more easily discriminated by listener panels than speech that is processed through hearing aids. Efforts to use a minicomputer to determine the auditory response to speech and speech-like signals, including pseudo-random noise, have been hampered by delays in obtaining key computer hardware. Finally, work has begun on the development of an impact noise generator for hearing loss studies.

Personnel:

Activities of Branch personnel have been numerous and varied. Many functions are interagency involving things like opinions on criteria documents (noise and microwaves especially), review of contracts and grant proposals to other agencies, and various committees. The following is an attempt to be comprehensive:

Dr. Adnan Akay has recently joined the Branch. He is presently involved in working on impact noise effects.

Dr. Reginald Cook: member, Interagency Noise Effects Panel; member, Committee of Hearing, Bioacoustics and Biomechanics of the National Research Council, National Academy of Science; member Working Group 83 on Noise Effects on the

Fetus; invited lecturer, seminars on effects of noise - courses on speech, hearing and acoustical engineering, UNC and NCSU.

Dr. Teruzo Konishi: member of Technical Committee, Physiophysiological Section, Acoustical Society of America.

Dr. Donald McRee: Adjunct appointment, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; NIEHS representative on Inter-departmental Radiation Advisory Committee (IRAC) on Biological Effects of Non-ionizing Radiation; Chairman of Scientific Information Exchange Working Group; representative for DHEW on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines (organized by ERDA); representative on American-National Standards Institute C-95 Committee on Safety Standards of Non-ionizing Radiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50002-04 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Ototoxicity of Methyl Mercury

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS
Others:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS
	Phillip J. Walsh	Radiation Physicist	LEB	NIEHS
	Alexandra Yankwich	Health Services Officer	LEB	NIEHS

COOPERATING UNITS (if any)

Research Resources Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the relationship between the distribution of methyl mercury in the cochlear fluids and the alteration of the cochlear potentials in guinea pigs. Animals are treated with methyl mercury chloride labeled with ^{14}C and/or ^{203}Hg. Radioactivity in the endolymph, perilymph, cerebrospinal fluid and whole blood is measured with a liquid scintillation counter. The stimulus-related responses as well as the DC polarization of the endolymph are recorded from the cochlea. The cochlear microphonic and action potential are suppressed by methyl mercury but the endolymphatic potential remains unchanged. No specific accumulation of methyl mercury is found in either the perilymph or endolymph.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: One group of guinea pigs was treated with a daily subcutaneous dose of methyl mercury chloride labeled with ^{14}C, with a total dose of 17 mg/kg of body weight for a period of seven days. In three other groups of guinea pigs, methyl mercury chloride with ^{203}Hg was given intravenously with total doses of 0.2 mg/kg, 1.7 mg/kg and 17 mg/kg of body weight, respectively. The cochlear potentials were measured in the basal and third turns with differential electrodes. Methyl mercury concentrations in the endolymph, perilymph, cerebrospinal fluid and whole blood were determined with a liquid scintillation counter.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year this project has been expanded to cover a more complete dose range. The results for all doses are as follows. (1) When animals received subcutaneous administration of 17 mg/kg (2.4 mg/kg per day) of methyl mercury chloride labeled with ^{14}C each day for a period of one week and were tested one day after the last treatment, the action potential in response to 6 and 8 KHz showed suppression. The cochlear microphonics were suppressed more markedly in the basal turn than in the third turn of the cochlea. The endocochlear potential was found to be in the normal range. The concentration of methyl mercury in the cochlear fluids was about 0.4% of the concentration of methyl mercury in the whole blood.

(2) When 0.2 mg/kg of $CH_3{}^{203}HgCl$ was injected intravenously, results showed that the methyl mercury level in whole blood dropped rapidly during the first day after injection. After this initial fast decrease, the blood level fell more slowly with a half time of about one week. The concentration of methyl mercury in the perilymph, endolymph and CSF rose during the first day. Their peaks appeared at the end of the first day. The initial decline was rapid and the concentration of methyl mercury in cochlear fluids and CSF appeared to fall slowly with a half time similar to that of the blood.

(3) When the dose of $Ch_3{}^{203}HgCl$ was increased to 1.7 mg/kg, the methyl mercury level in whole blood showed the initial rapid decline followed by the slow fall. The half-time was 8 days. The peak of the concentration of ^{203}Hg in the perilymph and CSF appeared about 32 hours after the injection. The concentration in the endolymph peaked about 48 hours after injection.

(4) When doses of the intravenous injection were increased to 17 mg/kg, the kinetics of methyl mercury uptake were similar to that observed with the lower dose of methyl mercury. However, the initial fast decline in the blood level was slower and peaks in the cochlear fluids and CSF appeared within 24 hours. The concentration after 10 days was 10^{-2} µgm/gm in the cochlear fluids and CSF.

(5) A double labeled experiment with $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ was carried out. The ratio of ^{203}Hg to ^{14}C was 0.5. The blood and CSF showed 0.44 and 0.48, respectively after seven days indicating that most of the mercury detected was in the form of methyl mercury. The concentration of methyl mercury in

the cochlear fluids did not show specific accumulation in either the perilymph or endolymph.

The major part of this project has been completed. The report is in preparation for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Clinical reports of methyl mercury poisoning shows sensorineural hearing loss. The knowledge obtained by this project is important for an understanding of the ototoxic effect of methyl mercury.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50005-04 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS
Others:	Phillip J. Walsh	Radiation Physicist	LEB	NIEHS
	Philip E. Hamrick	Senior Scientist	RRP	NIEHS
	Alexandra Yankwich	Health Ser. Officer	LEB	NIEHS

COOPERATING UNITS (if any)

Research Resources Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.85	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ł

The long range purpose of this project is to increase the understanding of the basic mechanisms of electrolyte transport in guinea pig cochlea under normal conditions and under the influence of physical and chemical agents. The flux of ^{42}K, ^{22}Na and ^{36}Cl from the perilymph to endolymph was measured by perfusing the perilymphatic space with artificial perilymph containing these radioisotopes. The endolymph actively takes up ^{42}K against the electrochemical gradient and extrudes ^{22}Na. ^{36}Chloride diffuses passively from the perilymph to endolymph. Ouabain and anoxia suppress the uptake of ^{42}K and extrusion of ^{22}Na. The concentration of -Cl in the endolymph was decreased and the uptake of ^{36}Cl of the endolymph was also suppressed by anoxia. The effect of exposure to intense noise is under the initial stage of investigation.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Stimulus-related cochlear potentials were recorded in anesthetized guinea pigs. The perilymphatic space was perfused with artificial perilymph containing ^{42}K, ^{22}Na or ^{36}Cl at the rate of 2 µl/min. The duration of the perfusion ranged from 5 to 90 minutes. Concentrations of ^{42}K and ^{22}Na in samples of the endolymph and perilymph were determined using a gamma-spectrometry system. Concentrations of -Cl were measured by a microtitration method and the activities of ^{36}Cl was determined using a liquid scintillation counter.

MAJOR FINDINGS AND PROPOSED COURSE: (1) There was only a small decrement of the cochlear microphonics (CM) during perfusion. The magnitude of the action potential of the auditory nerve (AP) showed temporary suppression during the first few minutes of the perfusion but showed full recovery. The average value of the endocochlear potential (EP) recorded at the time of collection of the endolymph was comparable with that obtained from non-perfused cochlea.

(2) When the perfusate containing ^{42}K and ^{22}Na or ^{36}Cl was introduced into the scala tympani of the basal turn and both scala vestibuli and tympani were perfused, the activities of these radioactive tracers were detectable in the perilymph of the scala vestibuli 5 minutes after the perfusion was initiated. When the scala tympani alone was perfused for a period of 20 minutes, concentrations of ^{42}K, and ^{22}Na and ^{36}Cl in the perilymph of the scala vestibuli were about 30% lower than those obtained from the scala tympani. When the scala vestibuli alone was perfused for 20 minutes, the concentrations of these radiotracers in samples taken from the scala tympani showed only a few percent of that in the perilymph of the scala vestibuli.

We examined the activities of ^{42}K and ^{22}Na in the perilymph of the scala vestibuli and tympani after perfusion of the scala vestibuli alone in animals in which the cochlear aqueduct had been surgically obstructed. The activities of ^{42}K and ^{22}Na in samples taken from the scala tympani remained lower than those in samples from the scala vestibuli. The rate of clearance of ^{42}K, and ^{22}Na and ^{36}Cl was much faster in the scala tympani than in the scala vestibuli.

(3) When the scala vestibuli and tympani were perfused, the ^{42}K concentration in the endolymph exceeded that in the perilymph within 5 minutes after the perfusion was commenced. As perfusion continued to 40 minutes, the concentration of ^{42}K was about 12 times greater than the concentration of ^{42}K in the perilymph. The transport rate constant for +K was 0.013 min^{-1} and the half-time exchange was 55.1 minutes, when the concentration of +K is assumed to be 150 mM in the endolymph and 5 mM in the perilymph.

The concentration of ^{22}Na in the endolymph was less than 1% of that in the perilymph, even if the duration of perfusion was prolonged to 60 minutes.

When the scala vestibuli and tympani were perfused for a period ranging from 10 to 90 minutes, the uptake of ^{36}Cl in the endolymph increased exponentially.

The rate constant for ^{-}Cl was 0.01 min^{-1} and the half-time exchange was 69 minutes.

In cases of separate perfusion of the scala vestibuli or tympani, the activities of ^{42}K and ^{22}Na in the endolymph did not show marked differences. However, when the scala vestibuli was perfused with artificial perilymph containing ^{36}Cl, the concentration of ^{36}Cl in the endolymph was higher than when the scala tympani was perfused.

(4) The average concentration of -Cl in the perilymph and endolymph was 124 and 128 mM respectively in non-perfused cochlea. When the perilymphatic space was perfused with artificial perilymph (Cl concentration 140 mM), the average concentration of ^{-}Cl in the perilymph and endolymph was 138 and 140 mM respectively.

(5) By adding $10^{-3}M$ to $10^{-4}M$ ouabain to the perfusate, the cochlear potentials were suppressed during perfusion and the EP was -10 to -20 mV at the time of collection of the endolymph. The concentration of ^{42}K in the endolymph was markedly decreased. The concentration of ^{22}Na increased, compared with that found in normal animals. The concentration of ^{-}Cl in the endolymph was 10 to 15 mM lower than that in the perilymph. The concentration of ^{36}Cl in the endolymph was also decreased.

(6) When the perfusion was carried out after a lethal dose of pentobarbital sodium was injected intravenously, the concentration of ^{42}K in the endolymph did not reach the level of ^{42}K concentration in the perilymph. The activity of ^{22}Na in the endolymph reached 20% of its activity in the perilymph after 60 minutes of perfusion. The average concentration of ^{-}Cl in the endolymph was 127 mM 40 minutes after perfusion. The average concentrations of ^{36}Cl was only 1.5%, when the average concentrations in the perilymph was taken as 100%.

(7) We examined the effect of noise on uptake of ^{42}K and ^{22}Na in the endolymph in 10 guinea pigs. They were continuously exposed for 10 days to an octave band of noise with a center frequency of 1 KHz and a sound level of 110 dB. The maximum output of the CM was greatly reduced after exposure of noise. The EP in those animals did not show marked deviation from that in non-treated animals. The concentration of ^{22}Na in the endolymph was comparable with that obtained in normal guinea pigs. The concentration of ^{42}K showed a large variation among the animals; in three animals the concentration of ^{42}K in the endolymph was about half of the value formed in control animals and in four animals it was about twice as high.

We plan to perform (1) determinations of concentrations of ^{+}K and ^{+}Na in the endolymph and perilymph using a helium glow photometer; (2) a systematic study of the effect of noise on electrolyte transport in the cochlea. (A small noise-exposure chamber has been completed recently. This will allow us to facilitate preparations of noise-exposed guinea pigs.); (3) Studies in an effort to reveal ototoxicity of diuretics which may occur as a result

of the disturbances of the dynamic equilibrium of electrolytes in the cochlear fluids; (4) monitoring of the brainstem evoked response with chronically implanted electrodes in awake animals during a period of exposure to noise or during treatment with ototoxic drugs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to electrolytes has been frequently hypothetized but the supporting data are rather limited. Present data suggest the possibility that transport of electrolytes across the endolymph-perilymph barrier is involved in pathophysiological processes underlying noise-or drug-induced hearing loss. These studies are a part of our efforts to increase our understanding of the disturbance of mechano-electrical transduction mechanisms of the inner ear under the influence of physical and chemical agents.

PUBLICATION

Konishi, T., Walsh, P.J. and Hamrick, P.E.: Permeability of cochlear partition to potassium and sodium ions. J. Acoust. Soc. Amer. 60: S79, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50006-03 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Responses of Listeners to Speech Recovered from the Inner Ears of Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Sanitary Engineer	LEB	NIEHS
	Teruzo Konishi	Visiting Scientist	LEB	NIEHS
	Charles Hamm	Physical Science Technician	LEB	NIEHS
Other:	W.G. Thomas	Director, Speech and Hearing Clinic	NCMH	UNC

COOPERATING UNITS (if any)

Speech and Hearing Clinic, N.C. Memorial Hospital, University of North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is an extension of Project Z01 ES 50006-01 in which signals were imparted into the ossicular chain by normal acoustic methods and by direct activation by piezoelectric type transducers, and the resulting inner ear responses electrophysiologically recovered and subsequently presented to listener panels in discrimination tests. The current project was designed so that listener panels could compare electrophysiologically recovered speech initiated by (1) normal acoustic presentation (2) processing through hearing aids (3) by direct activation by piezoelectric type devices. Further, since pilot projects results indicated the existence of differences between the hearing aid versus acoustic and PZT method of presentation which were not accounted for by word discrimination tests above. Therefore a new (listener) test paradigm including paired comparison quality ratings were instituted. Preliminary results indicate relative parity between the normal acoustic and direct ossicular chain activated (PZT) methods with both being superior to hearing aids. The differences appear to be dramatic in the quality rating category.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Electrophysiological techniques used for recovery of speech from cochleas of live, anesthesized guinea pigs.
2. Identical tape recordings of phonetically balanced word lists used for each method of processing through guinea pig ear.
3. Processed lists presented to listener groups at 50 dB HL for word discrimination and quality ratings.

MAJOR FINDINGS AND PROPOSED COURSE: No new findings were obtained during 1976-77 as other efforts took higher priority. Previous major findings are: No statistical differences exist between cochlea responses to normal acoustic and direct ossicular chain coupled speech in terms of word discrimination. Both exceeded hearing aid processed speech.

Speech quality differences are presently being evaluated. Preliminary results indicate little "quality" difference between the normal acoustic and piezo-electrically initiated speech but vast differences between both and hearing aid processed speech.

Existing data will be analyzed and published. One additional experiment is planned. It's purpose is to test the intelligibility of speech initiated by direct driving of the bony shell of the cochlea itself.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings clearly demonstrate that direct ossicular chain coupled means of transducing speech onto the auditory system offer vast improvements in fidelity and freedom from distortion over that achievable by conventional hearing aids. Provided the implantation problems can be solved, which appears reasonable at this point, the method offers hope that improvements can be achieved in hearing prophalaxis for some individuals.

In experimental applications with laboratory animals, the device offers the opportunity to impart precisely controlled complex signals into the ossicular chain by direct electrical/mechanical transduction thus bypassing the difficult-to-control electro/acoustical step.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50011-05 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Theoretical Basis of Dose-Response Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Phillip J. Walsh	Radiation Physicist	LEB	NIEHS
Other:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS

COOPERATING UNITS (if any)

Research Resources Programs

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to continue development of theoretical models of dose-response relationships for ionizing radiation at the cellular level and to begin the development of generalized conceptual schemes encompassing chemical, biological and physical agents. The current approach is to: (1) accumulate existing data for chemical, biological and physical agents on cell survival, mutagenesis and carcinogenesis that is suitable for dose-response analysis; (2) selection of models that can be applied to the data base in (1); (3) propose conceptual schemes within which further data can be accumulated to answer critical questions raised in (1) and (2).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: This project has included the development of a generalized theoretical model for the effects of ionizing radiation at the cellular level. A three-state model at the cellular level (normal cells, killed cells, altered cells) appears to encompass existing data and existing models on the effects of ionizing radiation. Such a model can be used to focus experimental work which, in turn, will lead to further model development. Questions to be addressed are: (1) whether, and at what level, models can incorporate effects of chemical, biological and physical agents; (2) whether paradigms for extrapolation can be proposed; (3) whether correspondence rules can be mechanistic (relate microevents to macroevents) or only phenomenological (macroevents only).

MAJOR FINDINGS AND PROPOSED COURSE: There appears to be no conceptual framework or theoretical model that can be used to provide guidance for investigation of environmental hazards in general. However, there exists extensive experimental data which has not been analyzed in terms of constructing a coherent theoretical model. Dosimetric concepts for chemical and biological agents are different from those for physical factors; particularly ionizing radiation, this is due to different interaction mechanisms that may require differnet conceptual models. It appears that the field of existing models (about 12-15) can be reduced to two or three with major similarities. Therefore a generalized theoretical approach with simplifying and synthesizing concepts appear warrented. It also appears reasonable to ask whether chemical and biological agents can also be incorporated into a general scheme. The generalized models developed here although applicable to other agents have been tested for ionizing radiation only. Experimental results of dose response to cell alteration, killing, repair and survival are well described by the models.

The proposed course is to terminate the project since the principal investigator is leaving the Institute.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Establishment of human exposure guidance is based upon extrapolation from existing data using theoretical models. It is expected that this will always be true. Thus confidence in extrapolation will increase in direct proportion to confidence in the basis of theoretical models.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50014-08 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Microwave Exposure Systems and Microwave Dosimetry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Donald I. McRee Research Physicist LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.2	0.9

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the objective of this project to develop exposure systems for bioeffects research and to develop and test techniques for measuring energy absorption. During the past year a waveguide system for exposing isolated neurons was fabricated and tested. Equipment for stimulating the nerves and measuring action potentials has been assembled. The 2450 MHz absorber-lined horn system has been altered so that exposure of pregnant mice from above can be performed. A wheatstone bridge circuit which will accommodate six small thermistor probes has been designed and built. These thermistor probes will be used to measure colonic temperature in mice while being exposed to microwave radiation.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to develop microwave exposure systems for biological material which have well-defined, field characteristics; and to develop dosimetric techniques for energy absorption. A thorough study of the research requirements on the biological effects of microwaves and techniques for exposing biological specimens have been completed. Based on these studies, exposure systems will be designed and developed which provide known electromagnetic field characteristics. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, and liquid crystals are some of the techniques which will be investigated. Other techniques to determine energy absorption will be evaluated as they become available.

MAJOR FINDINGS AND PROPOSED COURSE: It has been determined that for the exposure of small biological specimens such as cell systems and isolated neurons, waveguide exposure systems offer excellent characteristics. During the past year a waveguide system for exposing isolated neurons was fabricated and tested. The 2450 MHz absorber-lined horn system was altered so that pregnant mice could be exposed from above. A wheatstone bridge circuit which will accommodate six small thermistor probes has been designed and build for measuring colonic temperatures in mice while being exposed to microwaves.

Waveguide exposure systems will continue to be fabricated, calibrated, and operated during the next fiscal year. The development of a system to measure the electrical properties, dielectric constant and conductivity, of biological material will proceed. A small thermistor probe, 1 mm in diameter, with projected accuracy of $\pm$ 0.01°C and excellent response time has been developed by the National Bureau of Standards. This probe is said to have no interaction problems with microwave radiation. Walter Reed Army Institute has also developed a non-interacting thermocouple probe. We have requested one of these probes for testing in our laboratory.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of exposure system with well-defined field characteristics will enable the Institute to perform accurate quantitative studies on the effects of microwave radiation on biological systems at frequencies ranging from 1-10 GHz. Techniques to determine the amount of energy absorbed will provide the capability of evaluating the data in terms of thermal or specific microwave effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-04 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Microwaves on Neural Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
Other:	Howard Wachtel	Associate Professor	Duke U.	

COOPERATING UNITS (if any)

Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this project is to determine the effect of microwave radiation on neurological response. Isolated neurons such as the abdominal ganglion of the Aplysia, the sciatic nerves of frogs and the saphenous nerves of cats will be exposed to CW, pulsed and modulated microwave radiation in the power density range of 1-10 mW/cm^2. The effects of the microwave radiation on the strength-duration of a stimulus to produce an action potential, amplitude of the response, and conduction velocity will be investigated. The fatigue and recovery of the neurons under rapid, multiple stimulation will also be studied. A waveguide exposure system has been designed and built. Equipment for stimulation and recording of neural responses has been assembled. Baseline data on the sciatic nerve of frogs without microwave exposure have been accumulated and analyzed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the abdominal ganglion of the Aplysia, the sciatic nerve of the frog, and the saphenous nerve of cats will be exposed to CW, pulsed and modulated microwave radiation in the power density range of 1-10 mW/cm^2. The effects of the radiation on cell membrane potentials, action potentials and conduction velocity will be investigated.

More complex systems such as the exposed spinal cord of decerebrated cats will be exposed. These test systems will be used to investigate the effects of radiation on synaptic neurotransmitters.

MAJOR FINDINGS AND PROPOSED COURSE: A review of the literature indicates that the brain and central nervous system are sensitive to microwave radiation. In most studies, no explanation of the mechanism by which microwaves interact with nerve cells is specified. It has been determined that the best technique for exposing isolated neurons to microwaves, without interaction of the microwaves with the electrodes, is in a waveguide system. A waveguide exposure system has been designed and built. Equipment for stimulation and recording of neural responses has been assembled. Baseline data on the sciatic nerve of frogs without microwave exposure have been collected and analyzed.

Neural specimens will be placed in a CW, pulsed, or modulated microwave field. The nerves will be stimulated and the effect of the microwaves on action potentials, conduction velocity and signal transmission wil be measured. The strength-duration of the stimulation necessary to produce an action potential with and without microwave exposure will be an important endpoint. The fatigue and recovery of the nerves under rapid, multiple stimulation will also be studied. If effects are observed, the mechanism of interaction of the microwaves with the neural specimens will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The neurological and behavioral effects reported in the literature illustrate the need for significant effort in this area. At present, the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

PUBLICATION

McRee, D.I., Wyatt, R.H., Haseman, J.K., and Somjen, G.: The transmission of reflexes in the spinal cord of cats during direct irradiation with microwaves. J. Microwave Power 11(1): 49-60, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50016-06 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwave Radiation on Biological Materials at Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS
Other:	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Labarotory of Environmental Biophysics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and macromolecular level, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects. Biological systems employed in the study are: bacteria and bacteriophage, solutions of DNA, 2-4 cell stage embryos from mice, rabbit erythrocytes and rat lymphocytes. In these experiments, the temperature, humidity, and other various growth conditions have been carefully monitored in order to eliminate any effects not intrinsically caused by the microwave radiation. Results have shown very few differences between exposed and control samples that could not be explained by the thermal heating of the microwave radiation.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Various cellular and macromolecular systems are exposed to microwave radiation at carefully controlled temperatures and exposure levels. Much of the work has been at 37°C. Biological systems employed in the study are: bacteria and bacteriophage, solutions of DNA, 2-4 cell stage embryos from mice, rabbit erythrocytes and rat lymphocytes.

MAJOR FINDINGS AND PROPOSED COURSE: Studies by others have been made which suggest that "non-thermal" microwave fields (fields which do not significantly increase the temperature of the specimen) at various frequencies interact with intracellular components to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified and in many cases the exposure parameters are not known quantitatively. Many of the effects appear to be frequency dependent. For example, the growth rate of Escherichia coli has been shown by others to be enhanced or retarded depending on the frequency in the range of 65-75 GHz. In the past, when Escherichia coli and Pseudomonas aeruginosa were exposed in our lab to 2450 MHz microwaves at 60 mW/cm^2 and held at 37°C, no effects on growth rate were observed. Under similar conditions no effect was observed on the lysis time of bacteriophage T-2, or on the denaturation curves of DNA and no teratogenic effects were observed when mouse embryos were exposed. These studies have indicated that if there are "non-thermal" effects at 2450 MHz, then these effects are relatively small in the systems we have investigated. Polish and Russian scientists report effects on membrane transport in rabbit erythrocytes and stimulation of isolated lymphocytes to undergo transformation to a blastocyst-like cell. The study using rabbit erythrocytes has been repeated as nearly like the Polish work as possible. We were able to very closely duplicate the experiments since we had the opportunity to discuss the project with Polish scientists familiar with the work. Some of the parameters tested (such as K^+ efflux) were found to be highly temperature sensitive but no effects on membrane permeability were detected that were not due to temperature changes. During the past year our work with rat lymphocytes in tissue culture has shown that there is no significant difference in stimulation when cells are exposed for 40 hours or less to 5, 10, or 20 mW/cm^2 of 2450 MHz CW microwave radiation and in cells not exposed but held under similar conditions of temperature and humidity. These studies are being pursued using pulsed microwave radiation.

We plan to continue our study with lymphocytes since stimulation would suggest immunological effects with possible profound effects on animal systems. We will also study other systems in which sensitivity to microwaves has been reported or which are desirable to test systems for microwaves.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to carefully control the temperature of the specimen and be able to reproduce the exposure conditions. We have the capability to do this at NIEHS and can distinguish observed effects of microwaves from purely thermal effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50017-04 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)
Effects of 2450 MHz Microwaves on Embryonic Development, Immunology and Fertility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Philip E. Hamrick	Senior Scientist	RRP	NIEHS
Others:	J.P. Thaxton	Consultant	NCSU	
	C.R. Parkhurst	Consultant	NCSU	

COOPERATING UNITS (if any)
Research Resources Program
Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline)

At 5 weeks of age the juvenile quail were challenged with sheep red blood cells (SRBC) and the levels of anti-SRBC antibodies were determined. The levels of specific antibodies, determined four days after antigen challenges, were the same magnitude for both the exposed and control quail. Following this assessment of humoral immunity, the quail were sacrificed and the bursa of Fabricius and spleen were removed and comparisons were made of exposed and control birds. No significant alterations were seen in the microwave exposed group. Other groups of eggs were exposed to a power density of 5 mW/cm^2 for the first 12 days of development. After hatching the quail were allowed to mature and then were mated. Eggs for the matings were collected and hatched in a standard hatching incubator. After collecting over 1000 eggs in each mating group, it was found that the exposed males when mated with either the control or exposed females produced a 10 percent decrease in fertility. The exposed females when mated with the control males resulted in the same fertility rates as mating the control females with control males. Mating behavior studies were performed. No differences in the number of matings or mating attempts were observed between control and exposed quail.

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to determine the effects of 2450 MHz CW microwave radiation on the embryological development of Japanese quail and the subsequent growth, reproduction, and immunological response of the mature quail which had been exposed during the developmental period. Fertilized Japanese quail eggs were exposed to 2450 MHz CW microwave for various periods of embryonic development at power densities of 30 mW/cm^2 and 5 mW/cm^2. The effects of exposure on hatchability, gross deformities and hematological parameters were measured in the 2 day old quail. Fertilized eggs were also exposed to 5 mW/cm^2 for the first 12 days of development. The quail were kept to maturity and growth rates, egg production, fertility and immunological response were measured.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of the fertilized eggs to a power density of 5 mW/cm^2 for the first 12 days of development has produced no gross deformities or changes in hatchability when the temperatures of the exposed eggs are maintained at the optimum incubation temperature during exposure. Hematological studies on the two day old chicks have shown a statistically significant increase in hemoglobin and decrease in monocyte percentage. Organ weights were also measured in these two day old chicks, and no significant differences between exposed and control quail were found. These experiments were repeated, but after hatching the quail were allowed to grow to 5 weeks of age. The hatched chicks were allowed to grow to 5 weeks of age. At 5 weeks the juvenile quail were challenged with sheep red blood cells (SRBC) and the levels of anti-SRBC antibodies were determined. The levels of specific antibodies, determined four days after antigen challenges, were the same magnitude for both the exposed and control quail.

Other groups of eggs were exposed and the hatched quail allowed to mature. The quail were mated and eggs collected for fertility studies. It was found that the exposed males when mated with either the control or exposed females produced a ten percent decrease in fertility. The exposed females when mated with the control males resulted in the same fertility as mating the control females with control males.

At the end of 22 weeks, the treated and control quail were sacrificed, examined and organs weighed. The testes, spleen, bursa, adrenals, liver and heart weights were compared. No significant difference in the weights of the testes, adrenals, and heart were observed between the irradiated and the non-irradiated quail. No differences were found in the weights of the spleen and bursa of the irradiated male quail when compared with the non-irradiated male quail. However, there was a significant increase in the spleen and bursa of the treated females when compared to the untreated females. The weights of the livers of the exposed female quail were significantly smaller than the livers of the non-exposed. In approximately 75 percent of the treated females, one lobe of the liver was noticeably smaller than the other lobe.

Experiments to determine the cause of the decrease in fertility due to the

effects on the male quail will be continued. The analysis of the sperm data will be completed. Total RNA and DNA of the testis of the quail will also be determined. Pathological studies of the organ tissue will be performed to evaluate the effects on organs; particularly the liver, spleen, and bursa. Immunological studies will be accomplished by determining the ability of the 22 week old quail to produce antibodies in response to a challenge by foreign cell types.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of whether exposure to microwaves during embryonic development has an effect on the developmental process and function of the organism after maturity must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

PUBLICATIONS

McRee, D.I., and Hamrick, P.E.: Exposure of Japanese quail embryos to 2.45 GHz microwave radiation during development. Radiation Research, (In press).

Hamrick, P.E. and McRee, D.I.: Humoral immunity of Japanese quail subjected to microwave radiation during embryogency. Health Physics, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50019-02 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS
	Reginald O. Cook	Senior Sanitary Engineer	LEB	NIEHS
Others:	Charles W. Hamm	Physical Science Tech.	LEB	NIEHS
	Stanley Hutcheson	Consultant	CA	UNC

COOPERATING UNITS (if any)
University of North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.65	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ⊦

Results of behavioral and audiological tests with humans imply that ability to understand speech and other complex signals (particularly in a noisy listening situation) suffers loss from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a complex interaction between the various levels of the auditory nervous system and functionally different receptors of the auditory end organ play a vital role in "sharpening" the sensory process. The objectives and techniques employed are very similar to Project 50003-03 and the projects are being merged. The objective is to use the rapid signal analysis ability of minicomputers to determine the auditory response to speech and speech-like signals including pseudo-random noise under normal conditions and conditions of auditory fatigue. Auditory fatigue and recovery process of single nerve fiber to speech and speech-like stimuli will be studied. Due to delays in obtaining key computer hardware from the computer manufacturer, we were unable to complete programming and interfacing of the computer during the 1976-77 period. It is anticipated that the data collection process can begin in several months.

PROJECT DESCRIPTION

METHODS EMPLOYED: Minicomputer, signal analysis equipment, pseudo random noise, electrophysiological techniques, initial noise exposure would be band limited white noise.

MAJOR FINDINGS AND PROPOSED COURSE: Program is in initial stages, efforts so far have been concerned with programming and debugging the computer. Project will be approached as outlined above.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years, it has become clear that the ability of the cochlear transducer to respond to pure tones is not an adequate indicator of its ability to respond to the natural complex stimuli present in the real world. Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistant in spite of the fact that complex stimuli perceived in a noisy environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50020-02 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Optical Fiber Motion Detector for Auditory System Measures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Senior Sanitary Engineer	LEB	NIEHS
Others:	Adnan Akay	Visiting Fellow	LEB	NIEHS
	Charles W. Hamm	Physical Science Technician	LEB	NIEHS
	Teruzo Konishi	Visiting Scientist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

A convenient means for making audio frequency displacement measures of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL) has long been needed in the study of auditory distortion and hearing loss mechanisms. Reliable measures of the motion of the basilar membrane would resolve present uncertainties concerning non-linear plateau effects at high intensities and frequency resolution from high and low frequency slopes of the maximas which block confirmation of hearing theories at the intra cochlear mechanical level. Developmental work in the past year has led to the construction of very compact laser energized fiber optic levers with which instantaneous waveform visualization at the 10 Angstrom level can be accomplished on ossicular chain preparations; the electro optic technology has been advanced far enough to be released for general auditory research use. This optic lever system is competitive with interferometric and optical heterdynaming techniques in resolution and is an order of magnitude simplier and less expensive. For redevelopment effort will be focused on solving the problems posed by approaching the basilar membrane through the cochlea without destroying adjacent structures.

PROJECT DESCRIPTION

METHODS EMPLOYED: Physics, electro optics, optics (fibers), electronic engineering.

MAJOR FINDINGS AND PROPOSED COURSE: A theoretical analysis performed in our laboratory revealed that 1-10 angstrom resolution at a S/N ratio of one was possible under an optimized configuration. Prototypes are being built to determine whether this level is achievable in practice.

In development of projects which push the state of the art, the best approach is usually interative prototype development, where attempts are made to solve only one set of problems per state. It is proposed to follow this paradigm.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Inability to measure the actual displacement of the basilar membrane has been a stumbling block to understanding the intracochlear signal transduction mechanism. It is hoped that with this measuring scheme some of the missing data links can be filled.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50021-02 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Ultrastructure of Hair Cell-Nerve Juntion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS
	Robert Gulley		LNO	NINCDS
Others:	Reginald Cook	Senior Sanitary Engineer	LEB	NIEHS
	T.S. Reese	Head, Functional Neuroanatomy	LNNS	NINCDS
	Alexandra Yankwich	Health Services Officer	LEB	NIEHS
	G. Neises		LNO	NINCDS

COOPERATING UNITS (if any)
Laboratory of Neuro-otolaryngology and Laboratory Neuropathology and Neuroanatomical Sciences, National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

The purpose of this project is to study changes in the ultrastructure of the hair cells of the organ of Corti. The present project is designed to study the ultrastructural and electrophysiological changes of hair cells after alteration of the ionic concentration of endolymph in the chinchilla cochlea.

Perfusion of the scala media with artificial perilymph resulted in a progressive decline of the cochlear potentials but there was no clear increase in the number of vesicles associated with the presynaptic membrane.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Stimulus-related responses were recorded with differential electrodes in the basal turn of anesthetized chinchillas. The endocochlear potential was also recorded from the scala media. Using a microperfusion technique, the endolymphatic space was perfused with artificial perilymph or isotonic KCl solution over a period of 20 minutes. The amount of perfusate ranged from 3 to 5 µl. Immediately after perfusion the cochlea was prepared for EM study.

MAJOR FINDINGS AND PROPOSED COURSE: No new major findings were obtained during the past year. Previous major findings are included. Control animals were perfused with isotonic KCl solution. The electrical responses were not markedly affected by the slow perfusion of the scala media with isotonic KCl. The endolymphatic space was intact and there was no hair cell loss and no breakdown of intercellular junctions. The synaptic region was normal with no accumulation of vesicular structures near the synaptic region.

Animals perfused with artificial perilymph showed a progressive decline of cochlear microphonics and action potential during perfusion. The endocochlear potential decreased in magnitude as perfusion progressed. The number of vesicular structures in the synaptic region of the outer hair cell were increased but there was no clear increase in the number of vesicles associated with the presynaptic membrane.

Animals perfused with artificial perilymph and kept under relatively quiet conditions showed similar EM changes to those observed after perfusion with perilymph.

The changes observed are not consistent with any single hypothesis of synaptic function and only indicate that the synapses in the organ of Corti can be manipulated by experimental changes in endolymph compositon.

The major part of this project has been completed. Since we did not anticipate having a time to finish this project, it has been dropped.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanism involved in synaptic transmission at the hair cell-nerve junction has been understood. The results obtained through this project will give us significant information concerning the dynamic characteristics of the hair cell-nerve junction. This information is important for an understanding of functional changes induced by noise exposure or by ototoxic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50022-01 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of Impact Noise Generator for Hearing Loss Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Adnan Akay	Visiting Fellow	LEB	NIEHS
Other:	Reginald O. Cook	Senior Sanitary Engineer	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this project is to design and build an impact noise generator whose peak level, rise time, and decay can be experimentally manipulated so that the effects of these parameters on hearing loss can be studied in laboratory animals.

Lack of such a generator has greatly contributed to the paucity of controlled laboratory studies of impact noise effects on hearing. Because of the high instantaneous peaks and rise times of impact noise, it cannot be recorded and reproduced in the conventional manner with audio equipment.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The mechanism of impact sound generation is investigated in order to produce sound pulses with controlled amplitude and frequency content. A theoretical analysis of the peak levels, frequency content and time history emerging from several generation mechanisms was performed. Methods for building a generator embodying the selected mechanism were considered for their mechanical simplicity and reliability.

MAJOR FINDINGS AND PROPOSED COURSE: The generator is in the prototype fabrication and testing stage so there are no major findings to report at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fact that it is not possible to reproduce impact sounds using conventional audio equipment has resulted in few if any well controlled laboratory studies of hearing damage from a type of noise which pervades industry and society. There is strong suggestive evidence that high intensity short rise time peaks rather than steady state levels are the major contributor to hearing loss. Because the temporal summing characteristics of the human auditory system operate over a longer time span than that of impact sounds, preceived loudness is not proportional to peak level or to peripheral organ damage possibilities. This effort should make refined studies of hearing loss/impact noise parameters possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50023-01 LEB

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Review of Impact Noise, its Mechanism, Control and Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Adnan Akay	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

Center for Acoustical Studies, North Caroina State University

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of a review of the literature on impact noise. Its purpose is to increase the understanding of basic mechanisms of impact sound generation, impact noise control and to review the status of present and proposed damage assessment criteria resulting from impact noise exposure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Literature review and critical evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: The mechanisms of impact sound generation are classified as air flow ejected from the space between rapidly approaching surfaces, rigid-body radiation, radiation from surface accelerations, pseudo-steady state radiation and as a consequential source, radiation from material fracture. These methods have been investigated recently but the present theoretical tools are not sufficient to describe the impact sound radiation in detail.

Although hearing loss due to impact noise has been reported as early as 1700, the significance of high intensity fast rise-time impulse sound have been long overlooked. The present status of research results does not appear sufficient for an understanding of the parameters or to support standards for impact noise induced hearing damage assessment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effect of impactive impulse sounds on hearing have not until very recently been recognized as a probably major source of hearing damage. This review summarizes the available work on the subject and emphasizes the need to consider this long neglected phenomenon.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (UCLA)

TITLE: "Effects of 60 Hz Fields on the Mammalian Central Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: W. Ross Adey, M.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: October 1, 1975

CURRENT ANNUAL LEVEL: $99,994

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this research are to determine the effects of 60 Hz fields from high voltage transmission lines on the central nervous system of mammals.

METHODS EMPLOYED: Environmental field conditions similar to those found in the vicinity of high voltage transmission lines will be simulated. These field gradients will range from 1 to 2000 volts/meter. The effects of these fields on the biological rhythms in rats will be evaluated. The effects on the neuroendocrine mechanism in rats will be examined by using 17-hydroxysteriods, calcium, phosphorus, vanilmandelic acid and other urinary indicators of biorhythmicity. Changes in the neurochemistry of brain tissue of cats will be studied. These studies will include the measurement of both Ca^{2+} and amino acid effluxes from the cerebral cortex of the cat.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of rats to electric fields of 50 volts/meter, and 500 volts/meter have been completed. Activity measurements at 50 volts/meter showed no differences as compared to the controls. At 500 volts/meter an increase in activity of the exposed over the controls were measured. At both 50 volts/meter and 500 volts/meter an increase in urine output was measured with no increase in water intake. At 500 volts/meter no change in growth rate was observed, however, at 500 volts/meter a slower weight gain was measured. Thyroid and adrenal tissue weights were taken at the end of exposure. Average weights of wet adrenal tissue was higher (57.8 mg) for the 500 volts/meter series than in the control group (43.1 mg). However, no differences were detected in averaged wet thyroid tissue weights (19.0 versus 18.5 mg).

Exposure of rats to 100 volts/meter will be carried out during the next year. The effects on circadian rhythms will be determined by measuring and analyzing overall activity of the rats. The effects on neuroendocrine mechanisms will be monitored by analyzing the amounts of sodium, calcium, phosphorus, vanilmandelic acid, and 17-hydroxysteriods in the urine. Changes in the neurochemistry of brain tissue of cats will also be studied. These studies will measure the calcium efflux (Ca^{2+}) from the intact cerebral cortex of the cat.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The electric power industry is planning to construct high voltage transmission lines with voltages up to 1200 kV. In the vicinity of the lines, high electric and magnetic field gradients will be present. The health effects of these fields are presently unknown. This research will contribute to the determination of whether these electric fields do or do not constitute a health hazard. This project contributes to the mission of the Institute in evaluating the health effects of physical factors in the environment.

UNIVERSITY OF UTAH

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: Om P. Gandhi, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: June 28, 1976

CURRENT ANNUAL LEVEL: $98,194

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level exposure to 915 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long Evans male rats will be exposed to a CW plane wave microwave field at frequencies of 915 and 2450 MHz and power density of 5 mW/cm^2. The animals will be exposed for 8 hours a day, 5 days a week for 16 weeks. An eight week recovery period will follow the exposure period. The following measurements will be made:

1. Biochemistry - Biochemical analysis of the cholinesterase activity of the blood, the SH group in the blood, and ketosteriods in the urine will be measured.
2. Behavior - Rodent activity will be measured using a rodent activity wheel. Water and food intake will be measured. Rats will be trained to lever press for food reward on a FR-DRL multiple schedule. Total response and reinforcement for each schedule component as well as response during time out between each component will be recorded. Interesponse times on each schedule component will be recorded.
3. EEG - EEG will be recorded biweekly using epidural removable stainless steel electrodes. These recordings will be made while the microwave radiation is turned off.

MAJOR FINDINGS AND PROPOSED COURSE: Construction of radiation chambers and microwave field distributions have been measured and determined to be no greater than 10 percent. Training of ten rats on the multiple fixed-ratio-differential reinforcement at low rates (FR-DRL) schedule has been completed. Activity chambers have been built and tested and baseline data obtained. Problems with EEG recording cannula implants were initially encountered but have been standardized and is now functional. Bioessays for cholinesterase, sulfhydral groups, and corticosterone has been tested giving satisfactory results.

The exposure of rats to 915 and 2450 MHz microwave radiation to a plane-wave field will be carried out in the next year. All the biological endpoints tested in the objectives will be measured in both exposed and control groups.

These animals will be monitored for an eight week period after completion of exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The biological effects of long-term, low-level microwave exposure on the central nervous system and behavior is not known. Presently, only a small amount of research is being performed to evaluate the health hazards of this type of radiation. This research will contribute to the determination of whether these fields do or do not constitute a health hazard. This project directly applies to the mission of the Institute in evaluating the health effects of physical factors in the environment.

UNIVERSITY OF WASHINGTON

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTORS: Richard H. Lovely, Ph.D.
Arthur W. Guy, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE INITIATED: June 20, 1976

CURRENT FUNDING LEVEL: $129,867

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level, exposure to 918 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long-evans male rats, 80-90 days of age will be exposed to 915 and 2450 MHz circularly polarized microwave radiation at a power density level such that the specified absorption rate will be equivalent to that produced in a 5 mW/cm^2 plane wave field. The exposure will be 8 hours a day, 7 days a week for 4 months. Two month post-irradiation evaluations will be performed. Rats will also be exposed to 2450 MHz plane wave microwave field at a power density of 500 $\mu W/cm^2$ using the same schedule as that stated above. The following indicators of biological effects will be made:

1. Biochemistry - Serum content for sodium, calcium, phorphorus, PTH, SH groups and cholorinesterase activity of the blood and 17-ketosteriods in urine will be measured.
2. Behavior - Animals will be evaluated for free-operant and free-repertoire behavior. Animals will be observed in the irradiation chambers and their specific orientation to the field, sleep, grooming, sniffing, rearing, drinking, eating, etc., will be recorded.
3. Electroencephalography - EEG recordings will be made on the animals following the four month irradiation period.

In addition to the above endpoints, some behavioral teratology, cataract evaluation, and longevity data will be obtained.

MAJOR FINDINGS AND PROPOSED COURSE: Pregnant rats were exposed to 918 MHz CW microwaves at an incident power density of 5 mW/cm^2. The exposure was for 20 hours per day for the first 20 days of gestation. No changes in viable offspring or stillborns were observed. Trends toward accelerated development and accelerated eye opening in the exposed groups were noted. Growth rates are now being measured. At 90 days of age acquisition and extinction of conditioned shuttlebox avoidance response will be measured. If changes in behavior are observed, RNA hybridization studies of the CNS will be determined. If no effects on behavior is noted longevity studies will be performed.

Exposure of adult rats for 8 hours/day, 7 days/week are now in progress. These exposures will be at both 918 and 2450 MHz and 5 mW/cm^2 for a 16 week period. The biological indicators listed in the Methods Employed section will be evaluated. Exposures to 2450 MHz microwave radiation at a power density of 0.50 mW/cm^2 will also be performed. Behavioral tests and blood chemistries will be performed on the rats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Institute has, as part of its mission, the responsibility for studying the biological effects of physical factors on the environment. The potential health effects of low-level, long-term microwave exposure is not now known. With the increase of microwave radiation in the environment, it is important to determine the effects of microwave exposure. The most often reported effect in the literature is on the central nervous system and behavior. It is important that the Institute study long-term, low-level, effects since very little research at these conditions is presently being done in the United States.

LABORATORY OF ENVIRONMENTAL MUTAGENESIS

LABORATORY OF ENVIRONMENTAL MUTAGENESIS
Summary Statement

The primary objective of the Institute's program in mutagenesis is focused on evaluation and definition of risks to both human somatic and germinal tissue resulting from exposure to genetically active environmental chemicals. The Laboratory of Environmental Mutagenesis was organized late in FY72. Because of the worldwide shortage of resources for the rapid development of research in various problem areas associated with environmental mutagenesis, staff members have used a variety of approaches to accomplish its objective. These include:

1. Development of an intramural research program divided into eight main areas. Work on particular projects has been carried out by different mechanisms:

   a. in-house research by staff scientists
   b. support of work at other institutions in the collaborative research program with staff scientists as project officers
   c. developing an announcement in collaboration with the extramural program office to solicit grant proposals in specific emphasis areas of environmental mutagenesis

2. Development of a coordinated national program in environmental mutagenesis. A variety of mechanisms have been initiated including:

   a. participation in the DHEW Subcommittee on Environmental Mutagenesis (SEM)
   b. developing workshops and conferences in special emphasis areas
   c. developing collaborative programs with other institutes within NIH and with other agencies
   d. developing programs within the Environmental Mutagen Society in which staff members serve as officers, councillors or members
   e. developing collaborative studies on genetically active environmental chemicals to rapidly develop a data base to assist in evaluating the risk of exposure to man

3. Development of better communication and coordination at the international level. Staff members are associated with several international programs including:

   a. US-USSR Environmental Protection Agreement
   b. participation in international workshops and conferences
   c. collaboration with IARC (Lyon) on the mutagenicity of chemical carcinogens

4. Development of better mechanisms for information exchange. These include:

a. support of the Environmental Mutagen Information Center (EMIC)
b. serving as associate editor or members of the editorial board on journals which publish research in the area of environmental mutagenesis

5. Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at:

a. the predoctoral level
b. the postdoctoral level

INTRAMURAL RESEARCH PROGRAM

The intramural staff has been organized into seven programs. The major research accomplishments in each program are as follows:

The Microbial Genetics Section has made considerable progress in the development and improvement of simple and comprehensive tests for gene mutations and other types of genetic damage in microorganisms. Dr. Zeiger has attempted to correlate the mutagenicity of various procarcinogens using rat liver homogenate as activation system with measurement of the enzyme activity in the same aliquots. Together with Dr. Chhabra, he also is trying to study the relationship between the mutagenic activation in the intrasanguineous host-mediated assay and activation in vitro using bacteria as indicator organisms.

Drs. Frezza and Zeiger are using two different yeasts in the intrasanguineous host-mediated assay. Dr. Malling and Mr. Claxton have developed an instant computerized data acquisition system for the short-term testing of mutagenic activity.

Yeast is the only system for studying mitotic recombination. Unfortunately, most yeast strains are rather impermeable. Dr. Callen is using crystal violet to isolate more permeable strains of yeast. He is also studying the formation of toxic and mutagenic compounds by UV irradiation of fuel oil on top of water.

Dr. Scott has been working especially on developing a system in Aspergillus nidulans for detection of 50 plus genetic events. These events range from point mutations, chromosome aberrations, and mitotic recombination. Dr. Scott has tried also to develop Salmonella typhimurium, E. coli, Neurospora crassa, and Aspergillus nidulans. None of these systems are as sensitive as Tradescantia.

Mr. Harvey has studied the tritiated water as a mutagen in the ad-3 mutation system.

Dr. Ong has developed a spot test system for detecting reverse mutations at the ad-3 locus of Neurospora crassa. Various tester strains have been selected that exhibit sensitivity to various types of chemical mutagens. This rapid assay will complement the ad-3 forward mutation system earlier developed by Dr. de Serres which has been used by Drs. Ong and de Serres to test the mutagenicity of several chemical carcinogens and a wide variety of environmental chemicals. These studies are designed primarily as structure-function

analyses to determine the correlation between types of genetic damage induced by compounds of similar structure. Many chemicals do not exert mutagenesis by themselves but can be converted to mutagenic metabolites. Drs. Whong and Ong are using the ad-3 system to study the activation of dimethylnitrosamine to mutagens in the host-mediated assay and are comparing the results from different tissues and species.

Drs. de Serres, Ong, and Inoue also are continuing studies on repair-deficient strains of Neurospora. In a comparison between the normal heterokaryon and one homozygotic for repair-deficient mutants for five mutagens, it was found that they all caused more killing in the repairless strains than in the normal strain, but only those chemicals which react covalently with the DNA. Mr. Harvey also has extended the study on the repairless mutants in Neurospora and has shown that upr-1 and uvs-2 are not in the same pathway of repair.

Drs. Maier, Chhabra, and Malling have focused their attention on the activation systems which occur in the testicular cells and found that nitrofurazones are activated to mutagenic metabolites.

Great progress has been made within the Mammalian Biochemical Mutagenesis Program, where Dr. Soares was able to isolate biochemical mutations in mice using the Biochemical Specific Locus Mutation System. Dr. Soares used TEM as a model mutagen and has isolated more than 10 mutants. Dr. Valcovic has continued the study of the Co^{60} induced biochemical mutants. This mutation system will be expanded in the future to cover many more parameters. Dr. Lee is working on a system for detection of heat-instability mutations and mutations which affect specific activity of enzymes. Mr. Burkhart is developing the techniques and computer programs for the high speed centrifugal enzyme kinetic analyzer (MCFA). In order to understand the limitations of these techniques, Dr. Pegoraro is studying the naturally occurring polymorphic enzymes in mice.

The Biochemical Genetics Group is new and is a very important addition to the Laboratory of Environmental Mutagenesis. The group will study the enzymes and mutant enzymes in detail in order to elucidate the molecular mechanism of mutagenesis in mammals. Dr. Li will characterize the various isozymes and mutant proteins and correlate the amino acid differences and antigenic determinants of lactic dehydrogenase in mammals. Dr. Marciniszyn will concentrate on the sperm-specific lactate dehydrogenase in mammals, and Dr. Chen will characterize the mutant proteins from mammalian somatic cells.

The studies within the mammalian genetics program are concerned primarily with the detection of induced genetic damage in mice, investigations of the transmissibility of the damage to the progeny, and the long-term effect of mutations and chromosomal aberrations on the development of the embryo.

Drs. Sheridan and Soares are comparing the induction of dominant lethal mutations and translocations induced in a wide variety of inbred strains of mice after treatment with a given dose of triethylenemelamine. The objective of this work is to develop a battery of tester strains ranging from very sensitive to very resistant which will better mimic the range of variation that we can expect in the heterogeneous human population. They also are studying the induction of recessive lethal by chemical mutagens. This is an especially important study

since it can be assumed that each human carries 5-6 recessive lethal mutations, and not much is known about their effect on fitness of the individual. In order to study the apparent lack of response of mouse spermatogonial stem cells to mutagenic treatment, Drs. Sheridan and Soares have used combined treatments of radiation and chemical mutagens and are investigating whether or not pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemicals.

A study to detect induced genetic changes in both somatic and germ cells is being carried on by Drs. Soares and Sheridan. Both lymphocytes and sperm from chemically treated males are being examined for chromosomal aberrations. The aim of this approach is to use somatic cell mutagenesis as a predictor for germ cell effects. The success of this program could greatly reduce the cost and time of mammalian mutagenicity testing.

Drs. Burki and Sheridan have studied chromosome aberrations in early mouse embryos after mutagenic treatment of parents. While dominant lethal effects have been induced by several chemicals, the genetic causes have not been elucidated. This research seeks to determine the relative contribution of chromosome aberrations to this class of dominant lethal events.

Drs. Michelmann and Sheridan have studied the effect and unbalanced genomes on the development of the early embryos. These studies are especially important for understanding the Down Syndrome in humans.

In the Somatic Cell Genetics Group, the mechanism for induction of mutations in mammalian cells in vivo and in vitro are being sought. Drs. Malling and Ansari are utilizing the various hemoglobins of the inbred strains of mice which differ in as many as nine amino acids. Attempts to produce specific antibodies to these variant hemoglobins are carried out. The antibodies will be coupled with fluorescent molecules. Using these labeled antibodies, mutations can be detected in red blood cells. Other systems are being developed by Dr. Malling which are based on detection of mutations in sperm specific enzymes using histochemical techniques. At the present time we have developed histochemical stains for succinyl dehydrogenase, α-glycerolphosphate dehydrogenase, and lactic acid dehydrogenase and have found two inhibitors for the first and last enzyme. Mr. Dix is attempting to develop an automatic screening system for detection of these variant cells down to the level of 10^{-6}.

Dr. Huang is developing a gene mutation detection system in human diploid fibroblasts. Forward mutations will be detected at a sex-linked locus (HGPRT) and an autosomal locus (AGPRT). At the same time he is measuring the amount of adduct formed after reaction of the mutagen with the DNA. This will quantitatively give the efficiency of the chemical as a mutagen. Dr. Huang also is studying the effect of particular matter such as asbestos on cell death and mutation induction and has found that the toxicity is directly related to the amount of phagocytosis. In order to better understand the mutation expression, he is correlating the thioguanine resistant mutation frequency with the half-life of HGPRT in the cells.

A predominant part of the effort within the Population Genetics Program concerns itself with determination of the frequency of newly arisen genetic variation

and its effect on the fitness of natural populations. In order to provide the background, Dr. Voelker is studying naturally occurring alleles of enzyme loci which result in no enzyme activity at all. To do this he has constructed a strain which is heterozygotic at 22 loci.

Drs. Langley and Smith have extended this study to see if there is any association among the various biochemical polymorphism and the physiological or developmental effect of these genes. They found very little non-random association.

Drs. Langley and Shah have studied the variation of the mitochondrial DNA within the species of Drosophila; this will lead to a better understanding of the role of the mitochondria DNA in the cellular development.

The Mutagenesis Testing Program is a new function for LEM established by this year's budget, which will be carried out under the collaborative program and coordinated by Dr. Valcovic. While it is not part of the intramural research program, it can be considered to be an extension of the in-house effort.

Until recently, mutagenicity testing was done on preselected compounds in a manner in which the testing laboratories knew the identity of the substances under test and the "expected" results, i.e., positive for compounds selected because of their carcinogenicity and negative for food additives. There is no completed study in which substances were tested blind using a standardized protocol. Also, little attention has been placed on reproducibility and variability within and between laboratories. These aspects are currently under investigation in microbial systems by NCI but the results will not be available for 1-2 years.

In the NIEHS testing program a large number of substances will be tested in a blind study. This will involve the use of a hierarchy of tests proceeding from rapid screening in simple, highly sensitive microbial systems, through *in vitro* mammalian systems, up to whole mammal tests.

COLLABORATIVE RESEARCH PROGRAM

In the Collaborative Research Program most of the contracts fall naturally within five areas: (1) development of systems for monitoring of the human population for mutations, (2) development of risk extrapolation systems to predict induced mutation rate in the human population, (3) development of short-term test systems for mutagenicity, (4) monitoring of the human environment for mutagenic compounds, and (5) dissemination of mutagenesis information. The contracts will be described following this outline.

1. Mutation monitoring systems for the human population

In a contract at the University of Washington, an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing hemoglobin S rather than normal hemoglobin. Specific antiserum is being developed to detect mutations which produce hemoglobin S. By conjugating the antiserum with different colored fluoresent dye, mutant RBC's will fluoresce.

2. Risk extrapolation systems

a. Recessive lethal mutations. In a contract at The Jackson Laboratory progress has been made toward the development of a new mouse strain carrying multiple chromosome inversions to make it possible to detect gene mutations (recessive lethal mutations) in the chromosome regions covered by the inversions. One of the 39 inversions thus far recovered has been used in tests to detect x-ray induced recessive lethal mutations to demonstrate the general utility of this approach. Fourteen inversions have been mapped and are now being combined into a single mouse strain. Some of the combinations have lead to sterility.

b. Biochemical mutations. A contract with The Jackson Laboratory involves the incorporation of 13 additional mouse enzyme alleles into the C57BL/6J strain. This will directly complement the electrophoretic assay system developed at our Institute and will significantly enhance the sensitivity of the system to detect biochemical specific locus mutations.

In a contract with Research Triangle Institute, the progeny from treatment of the male parent with TEM is presently being screened with a role of 800 mice per month. Many different types of mutations have been found, such as esterase mutations, etc.

c. Polygenic mutations. A contract with the Georgia Institute of Technology attempts to develop a polygenic assay for the detection of point mutations in mice. By studying changes in the means and variances of characters determined by many genes, it should be possible to detect genetic changes which do not express major phenotypic effects.

d. Dietary deficiency effect on mutation rate. In a contract with Knox College studies are being conducted in Drosophila melanogaster to determine the effects of dietary deficiency on the sensitivity to mutagenic treatment. Preliminary results have indicated that deficiencies in choline and cholesterol increase the amount of genetic damage observed following treatment.

3. Short-Term Tests for Mutagenicity

a. Computer system for short-term tests. A contract at New York Medical School attempts to develop, in collaboration with DCRT in Washington, a computer system for assisting, advising, and collecting the data from short-term tests. The system is now working very well.

b. Testing of Neurospora mutants. We have continued to support a contract at Miles Laboratory to perform the genetic analysis of ad-3 mutants recovered from experiments on haploid strains of two-component heterokaryons. During the past two years we have also been using repair-deficient strains of Neurospora, as the genetic characterization performed in this manner has shown striking qualitative differences between wild-type and excision repair-deficient strains.

4. Monitoring of the Human Environment for Mutagenic Compounds

By interagency agreement, we are supporting base-line studies to determine the mutagenicity of airborne chemical pollutants and other gases used

used by industry. Various clones of the plant Tradescantia paludosa are being developed to select one particularly sensitive clone for assays of the genetic activity of weak mutagens. Field studies have been done in collaboration with NERC-EPA to compare the effects of exposure to the same airborne pollutants under laboratory and field conditions, and positive results have been obtained from some heavily polluted areas.

5. Collection and Dissemination of Mutagenesis Literature

During the past year we have continued to support EMIC (Environmental Mutagen Information Center) by interagency agreement at the Oak Ridge National Laboratory. This center has over 19,000 bibliographic entries in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

NATIONAL PROGRAMS

To develop better coordination in environmental mutagenesis and to provide perspective for the intramural scientific staff in problem definition and resolution in the rapidly developing field, a variety of mechanisms have been developed.

SEM - (DHEW Subcommittee on Environmental Mutagenesis). Intramural staff members have continued participation with at least 20-30 representatives from DHEW and other government agencies to develop better communication within DHEW as well as between other agencies with programs in environmental mutagenesis. The Panel meets monthly for discussion, and minutes are recorded to provide the basis for furthering communication within the various member institutes and agencies. A subcommittee was formed of SEM to develop the government's general position on mutagenesis testing. Dr. Valcovic served as Executive Secretary of this group and Drs. Malling and Zeiger were working members. During the development of the document, several open meetings were held at NIH. The document was finally turned over to Dr. Rall in April 1977. This is probably the most important part of the SEM work in FY77.

Workshops and Conferences: In collaboration with The Jackson Laboratory in Bar Harbor, Maine, Dr. Sheridan organized a workshop to "Design New Methods for Assessing Mutations in Mammalian Systems," which was held on October 6-8, 1976. The proceedings will be published in Genetics.

Collaborative Programs with Other Institutes within DHEW and with Other Government Agencies:

1. NCI - In collaboration with staff scientists at NCI, a collaborative research program has been persued to determine the correlation between carcinogenic and mutagenic activity of chemical carcinogens and non-carcinogenic structural analogs. This has also involved development of mechanisms to review and evaluate performance of the contractors as well as the planning of workshops to evaluate the accomplishments of the overall program. In collaboration with staff members of the In Vitro Carcinogenesis Program, new plans have been made to validate microbial and mammalian tests for mutagenicity for use as prescreens in the carcinogenicity test program.

2. FDA - In collaboration with staff scientists in the Bureau of Foods, NIEHS staff scientists have participated in the successful development and review of collaborative research programs to develop common methods for screening and computerizing data handling.

3. EPA - In collaboration with staff scientists in the Office of Pesticide Program, NIEHS staff scientists have participated in the development of collaborative research programs to perform mutagenicity tests on pesticides.

4. NSF and Other Agencies - Staff scientists have participated in the review of proposals for contract work and in site visits to select suitable contractors for research in the area of mutagenicity testing.

INTERNATIONAL PROGRAM

Staff members are associated with a wide variety of international programs with individual countries as well as with international agencies and programs.

US-USSR Environmental Protection Agreement - Drs. Langley and Valcovic visited several genetic laboratories in the Soviet Union in September 1976 to discuss future collaboration under this agreement. The fourth workshop on Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis was held in San Francisco, California, in April 1977. The program was focused on research directed towards developing better screening systems to detect mutagenic and carcinogenic activity as well as methods suitable for genetic monitoring of the human population.

Collaboration with IARC (Lyon) - During FY77 a new program was continued which involves participation between the National Institute of Environmental Health Sciences staff scientists, EMIC personnel and IARC scientists to translate mutagenicity data of known chemical carcinogens. Staff members also are participating in a series of workshops organized by the IARC staff to evaluate the correlation between carcinogenic and mutagenic activity and the possibility of using short-term tests for mutagenic activity to predict potential mutagenic and carcinogenic activity in man.

US-People's Republic of China - In connection with the expanded program of scientific exchange with the People's Republic of China, a Chinese delegation visited the Laboratory of Environmental Mutagenesis in September 1976.

International Symposia, Workshops, Meetings - Dr. Malling participated in the meeting of the German Environmental Mutagen Society, held July 1-2, 1976. The meeting of the International Environmental Mutagen Society will be held in Edinburgh, Scotland, in July 1977; and Drs. Malling, Valcovic, and Zeiger will participate. Dr. Malling will be a lecturer at the EMBO Course in Mammalian Genetics in Harwell, England, in September 1977.

INFORMATION EXCHANGE

EMIC (Environmental Mutagen Information Center) - The Institute has continued to support the work of EMIC to collect, organize, and disseminate information on environmental chemical mutagens. The Center not only processes requests for

information from various government agencies, but is now becoming well known abroad. During the past year, EMIC's data file reached 19,000 bibliographic entries.

Journals - Staff members serve as associate editors, assistant editors or members of editorial boards of Mutation Research, Cancer Research, Environmental Health Perspectives, The Journal of Toxicology, and Reviews in Genetic Toxicology. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

TRAINING PROGRAMS

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. During the past year, we have provided facilities for research for three predoctoral students from the University of North Carolina at Chapel Hill. At the postdoctoral level, staff scientists include 16 visiting fellows from ten foreign countries.

In conclusion, during the past year we have been successful in recruiting key personnel for our intramural program, have continued cooperative programs with other Laboratories in the Institute, have coordinated program development of the Laboratory with complementary programs in other Federal agencies, and have established lines of communication with newly developing scientific organizations for environmental mutagenesis abroad. Along with the organization of workshops and conferences, these approaches will be used to stimulate research and development in this rapidly developing area of environmental health science.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60003-04 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mutagenic Activity of Chemical Carcinogens in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Ong	Geneticist	LEM	NIEHS
Other:	F. de Serres	Associate Director for Genetics	OD	NIEHS

COOPERATING UNITS (if any)
Illinois State University, Normal, Illinois
Miles Laboratory, Elkhart, Indiana

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Recent studies on the mutagenicity of chemical carcinogens in various test systems have shown that there is a positive correlation between mutagenicity and carcinogenicity. Previous studies in Neurospora crassa show that carcinogenic compounds are mutagenic in N. crassa and that non-carcinogenic compounds are non-mutagenic. Our studies also indicate that potent carcinogens induce predominantly base-pair substitution mutations. It is necessary to extend our studies to determine whether chemical carcinogens cause similar spectra of genetic alterations both in wild-type and repair-deficient strains of N crassa. The ad-3 test system of N. crassa is used in this study. Conidia from a genetically marked two-component heterokaryon of both wild-type and repair-deficient strains are treated with chemical carcinogens. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. Based on the background counts and the number of mutants, the survival and the mutation frequency are determined. The ad-3 mutants isolated from forward mutation experiments are analyzed by a series of genetic tests.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The ad-3 test system of N. crassa is used in this study. Conidia from a genetically marked two-component heterokaryon of both wild-type and repair-deficient strains are treated with chemical carcinogens. The treated and untreated conidia are analyzed for the presence of ad-3 mutants. The ad-3 mutants isolated from forward mutation experiments are made homokaryotic for the adenine requirements. Their genotype and the complementation pattern of each mutant is determined by heterokaryon tests with various strains used as testers. Mutants are then tested by means of dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS AND PROPOSED COURSE : Both H12 (wild type) and H59 (uvs-2) were exposed to seven chemical mutagens. Five of the mutagens (betapropiolactone, ethyl methanesulfonate, methyl methanesulfonate, and N-methyl-N-nitro-nitrosoquanidine, actinomycin-D and aflatoxn B_1) cause more killing and ad-3 mutation in H59 than in H12. The two mutagens that do not covalently bond to DNA (actinomycin-D and 2-aminopurine) show no difference in their killing and mutagenic activities in the two heterokaryons. Studies on the mutagenic activity of chemical carcinogens in H12 and H59 will be extended to other compounds. These studies will be carried out under a contract with Dr. Herman E. Brockman at Illinois State University. Mutants induced by chemicals will be isolated and analyzed. The spectra of genetic alterations caused by chemical carcinogens both in H12 and H59 will be compared.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of chemical carcinogens in N. crassa might provide a system that could be used for screening carcinogenic chemical agents in the human environment. Furthermore, the studies on the spectrum of genetic alterations caused by chemical carcinogens, both in wild-type and repair-deficient strains of N. crassa, might provide information in regard to the relationship between carcinogenesis and mutagenesis of chemical agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60004-04 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of Simple Mutagen Test Systems in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Ong	Geneticist	LEM	NIEHS
Other:	J. Guthrie	Microbiologist	LEM	NIEHS
	W. Whong	Visiting Fellow	LEM	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The adenine-3 (ad-3) forward mutation system in Neurospora crassa is one of the best systems for studying mutagenesis in eukaryotic microorganisms. With this test system, recessive lethal mutations resulting from either chromosome deletions or point mutations can be recovered at the ad-3 region. The limitations of this test system are that one cannot screen for the mutagenicity of a large number of chemicals in a short period of time and that not every laboratory is equipped to use it. It is necessary to develop simple test systems in Neurospora so that both reverse and forward mutations caused by environmental agents can easily be detected. In our previous studies we have developed a spot test system in Neurospora using two ad-3A testers. Further studies are being carried out to determine: (1) whether these two testers can be used in plate and suspension tests, (2) whether mutagenicity of procarcinogens can be detected in the vegetative cultures of these testers, (3) whether mutants carrying double UV-sensitive markers are more sensitive to mutagens, and (4) whether mutants carrying a cell wall defective genetic marker are more sensitive to mutagens. Studies are also being carried out to develop both a para-fluorophenylalanine (PFPA) and a cyclohexamide (CH) resistant forward mutation systems.

PROJECT DESCRIPTION

METHODS EMPLOYED: In the plate test, conidia from 7-day-old vegetative cultures of ad-3A testers are harvested and 2 x 10^7 conidia in one ml of saline are added with 0.1 ml of chemical stock solution into 2 ml of cover medium. The mixture of conidia, cover, medium, and the chemical are poured onto a plate containing 20 ml of Fries minimal medium. Revertants are scored after plates are incubated at 30°C for 7 days. In the suspension test, conidia are treated with chemicals in pH 7 buffer. After 2 hours incubation at 30°C conidia are plated in minimal medium (for revertants) and in minimal medium supplemented with adenine sulfate (for survival). Colonies are counted after plates are incubated at 30°C for 7 days. In the induction of reverse mutation in vegetative cultures of the testers, conidia are incubated in medium containing procarcinogens. After 7 days incubation condia from these new vegetative cultures are assayed for the ad-3 revertants. In the forward mutation experiments, chemically treated conidia are plated in the medium containing either PFPA (0/3 mg.ml) or CH (4 ug/ml). PFPA and CH resistants are scored after plates are incubated at 30°C for 7 days.

MAJOR FINDINGS AND PROPOSED COURSE: Our major finding includes: (1) that the ad-3A testers used in the spot test can also be used in plate and suspension tests and (2) that the mutagenic activity of procarcinogens such as aflatoxin and DMN, can be detected in the vegetative cultures of the testers. In addition, we have established optimal conditions for the PFPA and CH resistant forward mutation systems. We are also in the process of selecting ad-3A testers which carry double UV-sensitive markers and testers which carry osmotic (cell wall defective) genetic marker. Future effort will include: (1) the incorporation of an in vitro activation system in plate and suspension tests; (2) the determination of whether or not the addition of UV-sensitive markers or an osmotic marker increases the mutagenic sensitivity of the testers; and (3) determination of whether PFPA and CH resistant forward mutation system can be used for the screening of the mutagenic activity of environmental agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a simple test system will provide a simple, economical and quick system in eukaryotes for the detection and evaluation of the mutagenic activity of chemical agents in the human environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60005-04 LEM

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mutagenic Activity of Antischistosomal Drugs and their Analogs in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. Ong Geneticist LEM NIEHS

COOPERATING UNITS (if any)

Miles Laboratory, Elkhart, Indiana

LAB/BRANCH

Laboratory of Environmental Mutagenesis

SECTION

Microbial Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Hycanthone, lucanthone, niridzaole, F30066 and some other antischistosomal nitrofurans are attracting wide concern because of their potential carcinogenic and mutagenic hazards to treated individuals in the human population. Our previous studies have shown that hycanthone, lucanthone and their indazole analogs are mutagenic in Neurospora crassa and these agents appear to induce predominantly multilocus deletion mutations. Our previous study also indicates that there is no correlation between mutagenicity and antischistosomal activity of hycanthone and its indazole analogs. It would be of interest to extend the studies on the mutagenic activity and the structure-function relationship of the other antischistosomal agents.

The ad-3 test system of N. crassa is used in this study. Conidia from a genetically marked two-component heterokaryon are treated with antischistosomal agents. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. Based on the background counts and the number of mutants, the survival and the mutation frequency are determined.

PROJECT DESCRIPTION

METHODS EMPLOYED: The ad-3 test system of N. crassa is used in this study. Conidia from a genetically marked two-component heterokaryon are treated with antischistosomal agents. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. Based on the background counts and the number of mutants, the survival and the mutation frequency are determined. The ad-3 mutants isolated from forward mutation experiments are made homokaryotic for the adenine requirements. The genotype and the complementation patterns of each mutant is determined by heterokaryon tests with various strains used as testers. Mutants are then tested by means of dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS AND PROPOSED COURSE: F30066, a commonly used antischistosomal drug, and other antischistosomal nitrofurans were found to be mutagenic in Neurospora crassa. Genetic analysis of mutants induced by antischistosomal nitrofurans induce predominantly base-pair substitution mutations. Studies on the relationship between mutagenicity and antischistosomal activity of nitrofurans showed that mutagenicity can be dissociated from antischistosomal activity. This project will be terminated in the near future.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since antischistosomal agents are widely used for the treatment of schistosomiasis in the human population, it is important to determine the potential mutagenic hazards of these compounds to the treated individuals. Studies on the relationship between structure, mutagenicity, and antischistosomal activity of antischistosomal compounds and their analogs might lead to a production of non-mutagenic compounds that can be used for the treatment of schistosomiasis.

PUBLICATIONS

Ong, T., Callen, D. F., Huang, S., Batzinger, R. P., and Bueding, E.: Mutation induction by the antischistosomal drug F30066 in various test systems. Mutation Research 48:37-42, 1976.

Ong, T.: Mutagenic activities of nitrofuran in Neurospora crassa. Mutation Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60006-03 LEM

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mutagenicity and Mutagenic Specificity of Chemical Carcinogens in UV-Sensitive Strains of Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Inoue	Visiting Fellow	LEM NIEHS
Other:	T. Ong	Geneticist	LEM NIEHS
	F. de Serres	Associate Director for Genetics	OD NIEHS

COOPERATING UNITS (if any)

Miles Laboratory, Elkhart Indiana

LAB/BRANCH

Laboratory of Environmental Mutagenesis

SECTION

Microbial Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Many studies, in bacteria as well as in other microbial organisms, have shown that some repair deficient strains are more mutable than the wild types. Studies with ad-3 test system of Neurospora crassa have shown that repair-deficient strains, upr-1 and uvs-2, are more mutable than wild type after UV or gamma ray irradiation. It has also been shown that the spectra of genetic alterations caused by UV in repair-deficient strains are different from that caused by UV in the wild type. It is of interest to study and compare the mutagenic sensitivity of repair-deficient and wild type strains to various chemical carcinogens and to compare the spectra of genetic alterations caused by these carcinogenic agents in the UV-sensitive and wild type strains.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The ad-3 test system of Neurospora crassa developed by F. J. de Serres is used in this study. Conidia from UV-sensitive strains or wild type are treated with chemical carcinogens. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. The isolated ad-3 mutants are made homokaryotic for the adenine requirement. The genotype and complementation pattern are determined by complementation tests.

MAJOR FINDINGS AND PROPOSED COURSE: Results of mutation induction experiments with 4-nitroquinoline-1-oxide (4NQO) indicated that uvs-2 and upr-1 strains are more mutable than the wild-type strain and uvs-6 strain is similar to wild type. In uvs-3, 4NQO cannot induce ad-3 mutants. Uvs-5 is only one-third as mutable as wild type. Present data show that the response of UV-sensitive strains to 4NQO seems to be similar to that of UV. Studies on mutagenicity and mutagenic specificity with other carcinogens will be performed in these repair-deficient strains, and the role of repair ability in mutagenesis and carcinogenesis will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study shows that the repair-deficient strains, upr-1 and uvs-2, are not only more mutable than wild type by UV or gamma ray irradiation but also more sensitive to the chemical carcinogens, MNNG and 4NQO. If these strains are also more sensitive than wild type to other chemicals, the use of repair-deficient strains would facilitate the detection of mutagenic activity of various environmental agents using Neurospora crassa.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60007-03 LEM

PERIOD COVERED
July 1, 1976 to December 30, 1976

TITLE OF PROJECT (80 characters or less)

The Relationship between Self-Inhibitors of Germination and Mutation Induction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Scott	Visiting Fellow	LEM NIEHS
Other:	L. Moustafa	Senior Staff Fellow	LET NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Recent studies with Aspergillus have shown that marked differences in induced mutation frequencies after apparently replicate mutagenic exposure are mainly due to the presence of varied amounts of self-inhibitors of germination in the conidium at the time of mutagenic treatment. With some mutagens, removal of these compounds has also affected the qualitative mutational response of thirteen genes which may be simultaneously studied in this organism, Aspergillus nidulans. It would be very interesting to determine whether such substances are also present in the conidia of Neurospora crassa and to investigate the effect these substances might have on the processes of mutation induction.

Such a program, it is hoped, will increase the confidence with which the mutagenic data obtained with fungal test systems can be meaningfully extrapolated to man. Isolation of inhibitory substances might also be very useful in extending the presumptive mutagenic treatment times which may be given to fungi in a host-mediated assay or microsomal activation system, hence increasing the detection capability of this organism.

PROJECT DESCRIPTION

METHODS EMPLOYED: Routine microbial and biochemical techniques have been employed in this investigation. The 2-thioxanthine and methionine suppressor mutation screening systems of Aspergillus, which detect forward mutation at about 22 gene loci simultaneously, plus the heterokaryon 12 system of Neurospora, were used to investigate the effect these compounds have on mutation induction.

MAJOR FINDINGS AND PROPOSED COURSE: This investigation has determined that a self-inhibitor of germination exists in Neurospora crassa. Removal of these compounds from the conidia of this organism, as previously shown in Aspergillus nidulans, is essential in order to obtain quantitative results after treatment with deleterious agents. Also, a partial isolation and chracterization of the inhibitor of Aspergillus has been achieved. This project was terminated during this year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the greatest difficulties facing the establishment of test systems for routine quantitative mutagenicity testing is the heterogeneity of the test organism. Any procedure which reduced this biological heterogeneity increases the confidence with which the mutagenic data obtained with a test system can be meaningfully extrapolated to man. By controlling the amount of possible germination inhibitory substances in Neurospora and Aspergillus conidia, the degree of heterogeneity of any mutagenic test system which uses these organisms is decreased. Isolation of the inhibitory substance might also be very useful in extending the treatment time which may be given to fungi in a host-mediated assay or microsomal activation situation, hence increasing the detection capability of the fungi. Finally, such a study should also lead to a greater understanding of the basic processes involved in mutation induction.

PUBLICATIONS

Scott, Barry R. and Moustafa, Laila A.: An observation on conidial germination lines of Neurospora crassa. Microbial Genetics Bulletin 40:16-17, 1976.

Scott, Barry R.: The effect of various "Magdala Reds" in inducing paramorphic colony growth in Neurospora crassa. Chem. Biol. Interactions 16:243-246, 1977.

Scott, Barry R. and Moustafa, Laila A.: The germination of diethyl ether prepared conidia of Neurospora crassa. Microbial Genetics Bulletin (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60008-03 LEM

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)
Mutagenic Activity of Furocoumarins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Scott	Visiting Fellow	LEM NIEHS
Other	W. Sheridan	Research Geneticist	LEM NIEHS
	E. Soares	Senior Staff Fellow	LEM NIEHS
	M. Pathak		Harvard Medical School

COOPERATING UNITS (if any)
Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Furocoumarins in combination with blacklight are being used as chemotherapeutic agents in the treatment of diseases in man such as Leukoderma and Psoriasis. These compounds are also commonly occurring in man's natural and artificial environment. The possible exposure of the human population to these compounds is, therefore, relatively high and cause for concern. Systematically, little information is known about the comparative mutagenic, carcinogenic, and teratogenic effects of the furocoumarins. It would be interesting to determine the comparative mutagenicity of one of the furocoumarins, 8-methoxypsoralen (8-MOP) with various combinations of blacklight in a variety of microbial systems and to determine the type of genetic alteration caused by 8-MOP in each region. In the later stages of the project, the investigation will be expanded to other furocoumarins in order to determine whether there is a relationship between the mutagenicity and chemical structure of the furocoumarins.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: In the absence of radiation these compounds do not appear to induce mutations in Salmonella typhimurium, Escherichia coli, and Aspergillus nidulans. In combination with radiation, 8-MOP is a potent mutagen; however, under normal testing procedures effects of these compounds would be detected. Both the monomeric and cross linking primary damage have been found to exhibit genetic activity. The latter inducing approximately double the number of mutants as the former. Preliminary findings would also indicate that at equal dose-molarity regimes the effect of 8-MOP > Psoralen > 5-methoxypsoralen in inducing a genetic effect. This project will terminate this year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of the furocoumarins are essential and important because some of the compounds are widely used for treatment of Leukoderma and Psoriasis in human populations. The relationship between various microbial test systems could provide information on the potential mutagenic hazard of 8-MOP in humans. Furthermore, studies on the mutagenicity and the chemical structure of the furocoumarins could lead to a better understanding of the mutagenicity and other biological activities of the furocoumarins; this might result in either the production of non-mutagenic compounds that could be used for the treatment of diseases in man or treatment conditions which would reduce the exposure risk.

The reason for expanding this study into one aimed at establishing screening systems in microbes and mice is that it is common knowledge in pediatrics that newborn infants are transparent to visible light due to the fact that they lack pigmentation. As a matter of fact, physical examination using transillumination is very common in neonatology. Hence, any undetected photosensitizing drug administered to these infants (or their breast-feeding mothers?) could result in an increased genetic risk in future populations.

PUBLICATIONS

Scott, Barry R.: Failure to detect a mutagenic activity of 8-methoxypsoralen (in the dark) in strains of Salmonella typhimurium and Escherichia coli. Mutation Research 35:213-220, 1976.

Scott, Barry R., Pathak, Madhu A., and Mohn, Georges R.: Molecular and genetic basis of furocoumarin reactions. Reviews in Genetic Toxicology 39:29-74, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60017-03 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mutation Induction in Human Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. Huang Senior Staff Fellow LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A system to detect mutations at the HGPRT locus in cultured, diploid human fibroblasts has been established in this laboratory. The carcinogen N-acetoxy-2-acetylaminofluorene has been studied and found to be mutagenic in human cells. Dose response in the induced mutation frequency was demonstrated. As judged by enzyme assays, most of the cells which grow in selective medium are true mutants. Because of the relevance of the cultured diploid human fibroblast and because so much is known about the biochemistry and genetics of the HGPRT locus (HGPRT = hypoxanthine guanine phosphoribosyl transferase), this mutation detection system is potentially useful for environmental monitoring.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The simplest explanation is that thioguanine or azaguanine (TG and AG) resistance results from a deficiency of the enzyme HPRT which is specified by a gene on the X chromosome. A million human skin fibroblasts were treated with NA-AAF for one hour. The treated cells were dispersed and 2.5×10^4 cells were inoculated in petri dishes. On day 4, selection using 3×10^{-5}M TG was initiated. The selective medium was renewed twice a week. The cultures were strained and TG^r (thioguanine resistant) colonies were counted or isolated on day 29. TG^r colonies were then propagated and the HGPRT activity was determined. The ability to use purine salvage pathways was also investigated for all the TG^r lines by growing them in azaserine and hypoxanthine.

MAJOR FINDINGS AND PROPOSED COURSE: The mutagenicity of NA-AAF has been studied using the TG resistance testing system. Four doses, 10^{-5}, 7.5×10^{-6}, 5×10^{-6} and 2.5×10^{-6}M NA-AAF, were used. NA-AAF was mutagenic to human fibroblast. A dose-response relationship for induced mutation frequency using NA-AAF was demonstrated. All the TG^r colonies obtained are true mutants (HPRT-deficient) and the resistant phenotype was maintained after several divisions in the absence of thioguanine. Thus, the change is stable. We propose to characterize this system further with regard to other agents and the nature of the genetic lesions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Lesch-Nyhan syndrome is a heritable disorder of purine metabolism in man. The responsible gene is sex-linked recessive. The primary product of the gene is the enzyme hypoxanthine guanine phosphoribosyl transferase which is defective or absent in this syndrome. With knowledge of this inherited genetic defect, a system using this locus can be established to monitor the induction of mutations by environmental agent.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60019-04 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Use of Male Germinal Tissue in the Detection of Mutational Events

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. V. Malling Laboratory Chief LEM NIEHS

COOPERATING UNITS (if any)
Environmental Biology & Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline)

The aim of this project is to develop systems for measuring the frequency of point mutations and DNA change in male germinal tissue. These systems would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa. At present enzyme histochemical methods are being developed to detect point mutations in male mice and three enzymes and their inhibitors have been characterized. These enzymes are α-glycerophosphate dehydrogenase, lactate dehydrogenase and succinate dehydrogenase. Preliminary work has started with the Axiomate scanning microscope to determine its usefulness in measuring the distribution of DNA content in a population of sperm. It is hoped that this instrument may prove useful in showing changes in the distribution pattern in sperm populations derived from mice treated or untreated with known mutagens.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Some enzymes in sperm can be detected readily by histochemical methods. The enzymes under study in this laboratory are α-glycerophosphate dehydrogenase, lactate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase and sorbitol dehydrogenase.

The principle of these studies is that, with the use of standardized staining procedures and variations in coenzymes and by using specific enzyme inhibitors, it should be feasible to detect active site mutations in the spermatozoal enzymes. These mutations will be estimated by counting stained and unstained cells with the aid of a Zeiss axiomat microscope connected to a PDP-12 Computer and a teleprinter output. Information will also be collected on magnetic tape for processing in the Institute's PDP-11 computer.

MAJOR FINDINGS AND PROPOSED COURSE: At present, it has been possible to standardize the staining technique to the extent that it is reproducible after repeated experimentation. Apart from the three enzymes described later, isocitrate dehydrogenase can now be reproducibly stained but no specific inhibitors have been tested. It has also been possible to develop a system of inhibitors specific for three of the enzymes being tested. They are listed below in accordance with the enzyme.

Enzyme	Inhibitor
1. α-glycerophosphate dehydrogenase	ATP Oxamic acid Malonic acid
2. Lactic dehydrogenase	Urea, Oxamic acid
3. Succinic dehydrogenase	Malonic acid

The future plans include testing out the present system to determine the spontaneous frequency of variant cells as well as the frequencies induced by various mutagenic agents. Other enzymes and their inhibitors will also be investigated and preliminary work will start on using the FACS-1 cell sorter to monitor the proportion of stained and unstained cells as well as the distribution of DNA content within populations of mouse sperm.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Development of this system is important, because it would permit the detection of mutational events within a single individual that could be used to monitor the human population. Besides, it would also enable us to detect the genetic consequences of exposure to mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60020-03 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Identifying of Chemical Compounds Activated by Germ Cells to Mutagens in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Maier	Visiting Fellow	LEM NIEHS
Other:	R. S. Chhabra	Staff Fellow	LP NIEHS
	R. M. Philpot	Senior Staff Fellow	LP NIEHS
	H. V. Malling	Laboratory Chief	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study is to identify compounds which induce point mutations, after metabolic activation by testicular tissues to mutagens in mammals. The topics are: (1) Screening of the literature for compounds which react with testicular tissue, (2) Development of protocols and determination of optimal conditions for the in vitro mutation experiments, (3) Characterization of the activation capacity of germinal tissue in vitro and (4) Correlation with a histochemical mutation system using sperm of male mice. As a result we found that AF-2 and nitrofurazone are activated in the soluble enzyme system and the microsomal fraction of rabbit testis-tissue.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The enhancement of the mutation frequency in the arg+ -system of E. coli 343/113 in a microsomal suspension test by the Nitrofuran AF-2 in the presence of rabbit testes homogenates was established previously. The present data show that a component in the soluble fraction of the testes homogenate interacts with nitrofuran and produces a product mutagenic in E. coli. This component has a low molecular weight and is also present in testicular tissue of other species. A correlative study between mutation induction and enzymatic reduction of AF-2 and nitrofurazone by E. coli homogenate indicates the linkage of these two processes. The additional comparison with the enzymatic reduction of the two nitrofurans by homogenates from rabbit liver and testes demonstrates qualitative similarities between the biochemical properties of the mammalian and bacterial enzyme system for nitrofurans. The enzymatic reduction in testicular tissue of compounds which are highly mutagenic in lower organisms seems to be linked to in vivo conditions in testes. In the future attempts will be made to identify the low molecular weight compound and to expand the number of compounds activated by the enzyme preparations from the testicular tissue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is of importance to future generations to identify compounds that cause point mutations in germinal tissue. A compound which is highly activated by testicular tissue homogenates and induces mutations in indicator organisms is likely to induce point mutations in sperm. Such systems as these will aid in preventing unnecessary future genetic hazards.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60021-05 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Electrophoretic Mobility Mutation Analysis in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Valcovic	Geneticist	LEM NIEHS
Other:	H. V. Malling	Laboratory Chief	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study of specific enzyme mutations using electrophoresis has the capability of detecting mutations that result in either a non-functional protein or a functional protein with altered electrical charge. Our objective is to develop a single mammalian test system that will detect both electrophoretic mobility (EPM) and inactive enzyme (IAE) mutations in the same loci. Two inbred mouse strains which differ with respect to nine enzyme loci have been selected for this system. Adult males of each strain were treated with gamma radiation and mated to unirradiated females of the other strain to produce F_1 hybrid offspring. Tissue samples are obtained surgically and electrophoresis is conducted on crude homogenates. While sampling is not yet completed, preliminary data indicate that the system is at least as sensitive in detecting IAE mutations as is the morphological specific locus system. Several mutants have been obtained and currently studies are underway to determine the histochemical nature of the induced mutations.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The ratio between mutations resulting in IAE-mutations and EPM-mutations can be estimated from the changes in the zymogram of heterozygous offspring from a cross between two inbred strains which differ in the electrophoretic patterns of several enzymes. In the simplest case let us assume the normal heterozygote will have three bands for a particular enzyme. A mutant induced in either parent can result in one of three different electrophoretic patterns: (a) the presence of any one band indicating that the mutant is inactive; (b) the presence of any two bands, one at the normal position for one of the parents and the other at any position indicating that the inactive enzyme will form an active aggregate with the wild-type enzyme; and (3) the presence of three bands, two with changed positions, indicating an EPM-mutation. Since case (a) and (b) indicate inactive enzymes and (c) an EPM-enzyme, a direct estimation of the ratio between IAE-mutations and EPM-mutations can be made.

We have selected two inbred mouse strains which differ with respect to nine enzyme loci. Additional non-variant bands will be apparent with some of the strains; and while the number of loci that these represent is unknown, EPM-mutations may be detected at these positions.

Since it is important to correlate this sytem with Russell's specific locus test in mice, the first mutagen treatment utilized was ionizing radiation. Adult males were irradiated and after a sterile period ranging from 4 to 11 months, they were mated to non-irradiated females. The animals received a total of 1000r ^{60}Co gamma rays delivered as two 500r exposures with a dose rate of 90r/min. separated by 24 hours.

Sampling of progeny consists of obtaining blood by orbital puncture and one kidney by surgery. Subsequently, electrophoresis is carried out on the red cell lysate and kidney homogenate from each animal. It is anticipated that approximately 3000 offspring from each of the crosses (DBA x C57; C57 x DBA) will be analyzed for mutations at each of the nine loci showing electrophoretic polymorphism.

MAJOR FINDINGS AND PROPOSED COURSE: The radiation experiment has been completed and data analysis is in progress. Seven electrophoretic variants were detected in the slightly more than 2600 animals tested from the DBA treated males. Three of these were found not to be genetic changes (either somatic effects or preparation artefacts). The dose of radiation employed resulted in almost complete sterility in the C57 males.

This radiation project has now been terminated and a new project using chemicals and modified procedures was initiated (see project number Z01 ES 60054-01 LEM).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of chemicals may increase the genetic load of the human population which may possibly present a health hazard. There are two approaches that may be considered to avoid such an event. First, use animal test systems to detect chemicals which are mutagenic and prohibit or limit their use. Second, use mutant alleles at selected loci in the human population and monitor these for several years to determine whether there is an increase in the frequency of these alleles. The system under development will be pertinent to both of these approaches.

It will survey a large number of different loci to minimize the cost of testing chemicals for potential mutagenicity; and since many of the enzymes are also found in the human population, it will also provide a base-line for the study of the mutation rate of electrophoretic mobility mutations in the human population. Furthermore, since most of the loci code for specific enzymes, purification and biochemical analyses can be employed to determine the nature of the mutational event, i.e., base-pair substitution and/or addition/deletion in the DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60023-03 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparison and Evaluation of Mutagenic Test Systems for Mass Screening Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Claxton	Biologist	LEM NIEHS
Other:	H. Malling	Chief	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.1	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study is to compare the usefulness and scientific reliability of several screening systems for mutagenicity. The work will compare a variety of bacterial systems, activation systems, and host-mediated assays. The microsomal system's format and data analysis are computerized in order to facilitate objective analysis.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Several known mutagens that have different mechanisms of genetic action and different physical and chemical characteristics are being tested in-house in each of the selected test systems. The parameters of each system are varied in such a manner as to standardize the system for screening conditions. A comparison is then made between the sensitivity, reliability, etc., of the various systems used. Included within the systems being tested are several bacterial systems, activation systems, and a host-mediated assay system.

MAJOR FINDINGS AND PROPOSED COURSE: The microsomal activation system has been investigated and a standardized protocol for general testing has been produced. The computerization of the microsomal test system is in its final stages of testing. Work on the standardization of a host-mediated assay has begun and a preliminary study of the micronucleus test has been initiated. Future efforts will involve the direct comparison of standardized systems and the development of proper statistical methods for data comparisons.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This evaluation and comparison will provide scientifically sound basis for large-scale screening and testing of environmental chemicals for mutagenicity. This study will also provide answers as to the feasibility of extrapolating results from one system to another.

PUBLICATIONS

Claxton, Larry and Barry, Patricia: Chemical Mutagenesis: An emerging issue in public health. American Journal of Public Health (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60026-04 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Comparative Sensitivities of Different Mouse Strains to Mutagenic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Soares	Senior Staff Fellow	LEM NIEHS
	W. Sheridan	Mammalian Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Earlier studies, utilizing irradiation as the mutagenic source have shown that the genome of the test organism is a major factor in the determination of its susceptibility to genetic damage. It is our purpose to investigate to what extent such genetically caused differences in sensitivity pertain to other mutagenic agents. We also want to know if the relative level of sensitivity of a defined genome to induction of mutations is constant for all agents or variable depending on the mutagen used. Preliminary screening of five inbred strains in our laboratory, using chemical mutagens indicate the existence of strain differences between these strains of mice. Future efforts will include retests of these strains plus additional strains. In addition, efforts will be devoted to understanding the underlying genetic causes of the observed differences.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: We want to accrue a battery of test strains, each showing a distinct level of sensitivity to mutation induction, covering a range from highly sensitive to highly resistant. These strains will be selected on the basis of induction of dominant lethal mutations and increases in the percent of morphologically aberrant sperm by known potent mutagens. They will then be challenged by a successive array of other known or suspected mutagenic agents to discover any changes in their relative levels of sensitivity.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary screening of five inbred strains has been made. There are indications of differences between some of the strains tested. These differences give promise of the success of the project; however, retests of these strains plus additional strains will be necessary before final choices of strains to be included in the test battery are made. Future efforts will be devoted to understanding the underlying genetic causes of differences in sensitivity to mutation induction and the consequences of combining the genetic materials of strains with divergent sensitivities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the gene pools of human populations by and large are extremely heterogeneous, one would expect a broad spectrum of reactions to a mutagenic agent in the environment. Restriction of testing to single, often inbred, strains of another mammal such as the mouse will not give indications on a wide scale of what might be the effects of such an agent on the human population. By determining the effects of a compound on a batter of strains representing a variety of genotypes and differing sensitivities, we will better be able to approximate what might be expected in a heterogeneous mammalian population such as man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60027-04 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Comparisons of Effects of Chemical Mutagens on Somatic and Germ Cells of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LEM NIEHS
Other:	E. Soares	Senior Staff Fellow	LEM NIEHS
	R. Sorg	Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project is designed to explore the feasibility of using somatic cells to screen for chemically induced chromosomal aberrations. The use of somatic cells for screening purposes would allow for rapid screening of large numbers of animals provided such techniques are reliable in the detection of aberrations. Included in this project are comparisons with respect to the efficiency of detection of chromosome aberrations in germ cells (testicular preparations and sperm morphology), cultured lymphocytes and bone marrow preparations.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lymphocytes cultured from offspring of treated males are prepared for cytological examination and karyotyping. Similarly, testicular sections and air dried preparations of testicular tissues as well as sperm from the vas and epididymis are examined for chromosome abnormalities and sperm head abnormalities respectively.

MAJOR FINDINGS AND PROPOSED COURSE: In the studies conducted thus far, chromosome preparations from cultured lymphocytes or bone marrow seem to be less reliable for use in the detection of aberrations than chromosomes prepared from testicular tissues. Lymphocyte preparations are most useful in those cases where the chromosomal aberration (translocation) results in the production of (1) small fragments, (2) metacentric chromosomes or (3) chromosomes with disproportionately large or small lengths are relative to the control karyotypes. In functionally sterile males suspected to be translocation carriers, we found upon examination of sperm that (1) actual counts of sperm were severely reduced and/or (2) as many as 90% of the sperm present were abnormally shaped. The mutagenic effects in mice of chemical agents will be tested. The mode of action and the long-term effects of induced, heritable chromosomal abnormalities will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although chromosome abnormalities are known to exist and be transmitted to following generations, very little is known of the long-term effects of such aberrations. Studies of the effects of heritable chromosome aberrations in mice will foster a better understanding of the mode of action and significance to the well being of the animal of such abnormalities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60028-03 LEM

PERIOD COVERED
July 1, 1976 to December 31, 1976

TITLE OF PROJECT (80 characters or less)
Genetic Effects on Early Mouse Embryos after Mutagenic Treatment of the Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Burki	Visiting Fellow	LEM NIEHS
	W. Sheridan	Research Geneticist	LEM NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this project is to study the underlying causes of induced embryonic mortality in the dominant lethal test in the mouse. This is achieved by direct observation of the in vitro development of embryos and by cytogenetic analysis of their karyotypes. One of the most widely used tests of mutagenicity in mammals is the dominant lethal assay. While this system allows detection of induced increases in foetal mortality, the underlying causes are not clearly delineated. One of the proposed factors has been the induction of chromosomal aberrations. It is our purpose to investigate the processes and causes of foetal mortality by direct observation of the development of embryos and by cytogenetic analysis of their karyotypes.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The methodologies of culturing mouse embryos *in vitro* up to the blastocyst stage and on to the outgrowth of the trophoblast are used. Male mice are treated with a mutagen (TEM) and mated to untreated females to allow *in vivo* fertilization. Approximately thirty-two hours post mating, the two-cell embryos are retrieved from the oviducts of the females and established in cultures. An air-drying method is used to make cytological preparations from *in vivo* and *in vitro* grown embryos at various stages of development.

MAJOR FINDINGS AND PROPOSED COURSE: Treatment of the males did not reduce the rate of fertilized and cleaving eggs but affected further development of the embryos at all stages. The percentages of cultures embryos showing normal development decreased with increasing dose of TEM injected and varied with the time of conception postinjection for treated post meiotic stages of spermatogenesis. The evaluation of the cytological preparations is under way. After treatment of germ cells, the specific pattern of morphological and cytological aberrations of early mouse embryos induced by different known chemical mutagens will be compared. The *in vitro* culturing of embryos will also be used to observe directly the development of embryos carrying a known inherited mutation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: After treatment of germ cells, the specific pattern of morphological and cytological aberrations of early mouse embryos induced by different known chemical mutagens will be compared. The *in vitro* culturing of embryos will also be used to observe directly the development of embryos carrying a known inherited mutation.

PUBLICATIONS

Burki, K., Sheridan, W. and Soares, E. R.: *In vitro* embryo culture and chemical mutagenesis. Mouse News Letter, February 1976 (abstract).

Burki, K. and Sheridan, W.: *In vitro* culture of mouse embryos after TEM-treatment of the fathers. Seventh Annual Meeting of the Environmental Mutagen Society, March 1976 (abstract).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60033-02 LEM

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)
Simultaneous Detection of a Variety of Genetic Events in Aspergillus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: B. Scott Visiting Fellow LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

At present there is no system which can simultaneously detect both forward mutation and chromosome aberrations. This program is designed to fill this lacuna. A system has been developed in Aspergillus nidulans in which at least 50 plus genetic events can be detected. These events range from point mutations, chromosome aberrations, and mitotic recombination.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The basic linkage groups for each of the 16 linkage groups comprising the final heterozygous diploid have been constructed. Preliminary investigations with one of these linkage groups, in diploid conidia, has indicated that a large number of genetic events are detected at a high level of survival. This project will terminate this year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Overall at least 50 plus genetic events should be detected by the above system. These events range from point mutation to chromosome aberrations and other genetic events, by a single initial detection method. At the most simplistic level such a system could be a very powerful tool in detecting deleterious agents inducing genetic changes. While at the most advanced level it should be possible to determine whether or not there is a correlation between the various types of possible genetic alterations ranging from point mutations to viable chromosome aberrations and other genetic events which are important to mankind.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60034-02 LEM

PERIOD COVERED
July 1, 1976 to December 30, 1976

TITLE OF PROJECT (80 characters or less)
Detection of Air Pollutants and Low Levels of Mutagens/Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Scott	Visiting Fellow	LEM NIEHS
Other:	S. Lamm		Brookhaven Nat'l. Lab.
	L. Schairer		Brookhaven Nat'l. Lab.
	P. Klotz		Brookhaven Nat'l. Lab.
	A. Sparrow		Brookhaven Nat'l. Lab.

COOPERATING UNITS (if any)
Brookhaven National Laboratory, Upton, New York

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

"Low, repeated doses of such common place pollutants as fumes in the air and chemicals in drinking water are increasing risks of cancer, heart disease, and genetic mutations," according to the library of congress report. This report indicates also that the existing testing methods are expensive, arduous, and not sensitive enough to detect polluting agents. The primary aim of this project is to attempt to develop inexpensive biomonitors to detect these hazardous environmental agents and compare the results obtained with these systems with the work already in progress at Brookhaven using Tradescantia.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Established screening systems which detect mutagens have been employed in the first part of the investigation. These systems employ the organisms, Salmonella typhimurium, Escherichia coli, Neurospora crassa, and Aspergillus nidulans.

MAJOR FINDINGS AND PROPOSED COURSE: None of the above microbial test systems were capable of detecting the mutagenic activity of 1,2 dibromoethane (EDB) in the gaseous state at 50 to 100 X the concentration which doubles the spontaneous level of mutations in Tradescantia stamen hairs.

These apparently diverging results were experimentally explained by observations that EDB is capable of being converted to a mutagen in two ways, one involving a "breakdown" product in vitro (hence the previously reported mutagenic activity in the plate tests) and the other involving metabolic activation by plants. Associated with this study the methodology for in vitro qualitatively detecting promutagens which are converted by plant metabolism to active mutagens has been established. This project was terminated during this year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evolution of inexpensive biomonitor to detect hazardous environmental agents which induce genetic damage after chronic exposure to mutagens is urgently required. Such monitors once evolved could be routinely used to screen for air pollutants, act as early warning systems, and possibly be used to identify the deleterious agents involved.

PUBLICATIONS

Scott, B. R., Sparrow, A. H. Schwemmes, S. S., and Schairer, L. A.: Plant metabolic activation of 1,2 dibromoethane (EDB) to a mutagen of greater potency. Mutation Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60035-02 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Development of Test Systems in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Callen	Visiting Fellow	LEM NIEHS
Other:	R. Philpot	Senior Staff Fellow	LP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Attempts have been made to modify or generate new mutation test systems in Saccharomyces cerevisiae which include: (1) isolation of mutants in yeast which have an increased permeability to mutagens, (2) study of the ability of yeast to metabolize promutagens to genetically active products within the yeast cell and in the absence of mammalian liver homogenates.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) Presumptive permeability mutants were isolated by selecting colonies sensitive to crystal violet after growth of a mutagenized haploid strain. (2) Cytochrome P-450 was assayed in yeast cell suspensions sampled from a culture growing on medium containing glucose as carbon source. Log phase cells were treated in suspension with promutagens and the genetic activity assayed by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A number of haploid strains have been isolated which have properties suggesting that they possess mutations affecting the integrity of the cell membrane or cell wall. Some strains exhibit greatly increased sensitivity to ICR-170 when compared to the parental haploid strains. These strains are being further tested with other mutagens. A crossing program is being initiated to incorporate these genes in the diploid yeast strains which are routinely used in testing. (2) The promutagens β-naphthylamine, ethyl carbamate, dimethyl nitrosamine, and cyclophosphamide were found to be genetically active when incubated with log cells harvested from medium containing glucose. Yeast cells which did not possess cytochrome P-450 were not able to metabolize these compounds to genetically active products. Further compounds are being tested to evaluate the extent yeast can metabolize promutagens.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Short-term mutagenicity tests with a particular environmental agent are more reliable when data has been derived from a variety of organisms. Yeast has the added advantage over bacterial systems that it is a eukaryote and; therefore, the cellular and genetic responses to mutagenic agents may more closely resemble those of higher cells.

The yeast strain D4 is used to monitor gene conversion induced by mutagenic agents but this system is relatively insensitive when compared to test systems in some other organisms. Thus, yeast would play a more important role in short-term testing if the sensitivity of this strain could be increased. The activation system using log phase yeast cells is particularly convenient when compared to other activation systems since liver homogenate or an NADPH generating system is not necessary. This yeast system may more closely model the actual *in vivo* situation than systems in which liver homogenate is used.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60036-02 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetic Activity of Environmental Agents in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Callen	Visiting Fellow	LEM NIEHS
Other:	T. Ong	Senior Staff Fellow	LEM NIEHS
	R. Larson		Stroud Water Res. Center

COOPERATING UNITS (if any)

Stroud Water Research Center, Avondale, Pennsylvania

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Studies with yeast indicated that (1) a drug, F30066, used in China for the treatment of schistosomiasis, was genetically active and (2) the irradiation of fuel oil with UV irradiation of similar intensity and wavelength to that of sunlight resulted in the production of highly toxic and in some cases genetically active water soluble compounds.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) F30066 was genetically active in yeast. For example, in one experiment F30066 at a concentration of 13·3 μg/ml was found to result in a 6-fold increase over the spontaneous conversion frequency at a cell survival of 30%. (2) Exposure of the yeast strain D4 to fuel oil irradiated with UV for 12, 24, 48 and 96 hours resulted in a rapid rise in toxicity with increasing times of UV irradiation. Genetic activity was found associated with the 12 and 24 hour samples. With increasing times of irradiation the concentration of organic hydroperoxides rises rapidly. Individual organic hydroperoxides are being evaluated for any associated genetic activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: (1) The antischistosomiasis agent F30066 is used in China. These results suggest that this compound may be a genetic hazard. Continued use of this drug in treating the human population would be inadvisable without further evaluation of its genetic and carcinogenic hazard. (2) The irradiation of fuel oil samples simulated the effects of sunlight on an oil slick from a spill. These experiments establish that highly toxic compounds are produced which may be hazardous for sea life since they are also water soluble. Some of these compounds are also genetically active.

PUBLICATIONS

Ong, T., Callen, D. F., Huang, S. L., Batzinger, R. P., and Bueding, E.: Mutation induction by the antischistosomal drug F30066 in various test systems. Mutation Research 48:37-42, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60037-02 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Genetic Activity of Aflatoxins in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Callen	Visiting Fellow	LEM	NIEHS
Other:	T. Ong	Geneticist	LEM	NIEHS
	R. Philpot	Senior Staff Fellow	LP	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Aflatoxins are highly carcinogenic substances and can occur as contaminants in food. (1) Since aflatoxin B_1 has been shown to be a more potent carcinogen than aflatoxin G_1, it is of interest to compare their genetic activity in yeast. (2) The active metabolite of aflatoxin B_1 is thought to be an epoxide which can be converted to a dihydrodial by epoxide hydrase, an enzyme present in liver homogenate. 1,2-epoxy-3,3,3-trichloropropane (TCPO) is an inhibitor of the epoxide hydrase and therefore addition of this inhibitor to the mixture of aflatoxin B_1 and liver homogenate should increase the proportion of available epoxide which can react with indicator cells. (3) Yeast cells harvested from log phase cultures grown on medium containing glucose possess cytochrome P-450 while cells harvested from log phase cultures grown on medium containing galactose possess negligible cytochrome P-450. We are comparing the metabolism of aflatoxin B_1 to genetically active compounds in cells grown on glucose and in cells grown on galactose.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) Aflatoxin B_1 at a concentration of 100 µg/ml increased the ade convertant frequency from $3{\cdot}3/10^5$ survivors to $93{\cdot}0/10^5$ survivors. In contrast aflatoxin G_1, even at 200 µg/ml, did not significantly increase the conversion frequency. Therefore, aflatoxin B_1 has a greater genetic activity than aflatoxin G_1. (2) The inhibitor TCPO was found to be genetically active per se. However, in the presence of liver homogenate this activity could be eliminated, presumably due to binding of TCPO to proteins in the homogenate. When inhibitor was added to liver homogenate and aflatoxin B_1, there was an increase in the conversion frequency with increasing concentrations of inhibitor. Although these results may be considered to support the suggestion that the active metabolite of aflatoxin B_1 is an epoxide, alternative explanations are also possible since the inhibitor was also genetically active. (3) Aflatoxin B_1 could be metabolized to genetically active compounds by log phase cells harvested from either glucose or galactose medium. These results can be interpreted as evidence that in yeast aflatoxin B_1 can be metabolized by enzyme systems other than the mixed function oxygenases.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results provide additional evidence to support the observed correlation between mutagenicity and carcinogenicity. The effect of the inhibitor, TCPO, may support the idea that an epoxide is the active metabolite of aflatoxin B_1. The observed genetic activity of the inhibitor, TCPO, suggests that mutation experiments in which this compound is used as an enzyme inhibitor need to be interpreted with some caution. Results with yeast suggest activation of aflatoxin B_1 may not always proceed via the mixed function oxygenases. Whether this can also occur in other systems is not known.

PUBLICATIONS

Callen, D. F., Mohn, G. R., and Ong, T.: Comparison of the genetic activity of aflatoxin B_1 and G_1 in Escherichia coli and Saccharomyces cerevisiae. Mutation Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60038-02 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Aflatoxin Activation and Mutagenesis in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Ong	Senior Staff Fellow	LEM NIEHS
Other:	J. Guthrie	Microbiologist	LEM NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Aflatoxin B_1 is one of the most potent chemical carcinogens found in the human environment. This and other aflatoxins are common contaminants in agricultural products. Our previous studies have shown aflatoxin B_1 and G_1 can be converted by hamster and mouse liver homogenates to metabolites which are mutagenic in Neurospora. Our studies also showed that addition of the epoxide-hydrase ~~inhibitor,~~ 1,1,1-trichloropropane-2,3-oxide, to the test system increased ad-3 mutation frequency. Further studies using the ad-3 test system of Neurospora are carried out to determine whether (1) aflatoxins can be converted by rat liver homogenates to metabolites mutagenic in Neurospora, (2) more mutagenic metabolites are being converted from aflatoxin B_1 than G_1 by rat liver homogenate, (3) more mutagenic metabolites are being converted from aflatoxin G_1 than B_1 by the kidney homogenates of male rats, (4) kidney homogenates from female rats are failing to convert aflatoxin B_1 and G_1 to mutagenic metabolites, (5) NADPH is necessary for the conversion of aflatoxin to mutagenic metabolites and (6) pretreatment of mammals with phenobarbital increases the conversion capacity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Conidia from a genetically marked two-component heterokaryon homozygous for excision-repair deficient strain (H59) are harvested and treated with aflatoxin B_1 and G_1, homogenates from either rat liver or rat kidney and a NADPH-generating system at 37°C for 2 hours. At the end of the treatment conidia are washed. Treated and untreated conidia are assayed for the presence of ad-3 mutants by the direct method. Mutants isolated from these experiments will be characterized by a series of genetic tests.

MAJOR FINDINGS AND PROPOSED COURSE: Major findings can be summarized as follows: (1) the homogenate of liver and kidney from either male or female rats can all convert aflatoxin B_1 and G_1 to metabolites mutagenic in Neurospora, (2) aflatoxin B_1 is much more mutagenic than aflatoxin G_1 in an in vitro activation system using liver or kidney homogenate, (3) NADPH does not appear to be necessary for the conversion of aflatoxin B_1 to mutagenic metabolites, and (4) pretreatment of rats with phenobarbital does not enhance the mutagenic activity of aflatoxin B_1 in an in vitro activation test system. In the future, attempts will be made to identify the active metabolites of aflatoxins by either genetic or biochemical studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aflatoxins are common contaminants in agricultural products. These compounds, therefore, are very closely related to human health. Studies described in this project will provide information in regard to the potential mutagenic hazard of these mycotoxins to man, in regard to the mechanism of aflatoxin mutagenesis, and in regard to the relationship between carcinogenesis and mutagenesis of these compounds.

PUBLICATIONS

Matzinger, P. K. and Ong, T.: Mutation induction of rodent liver microsomal metabolites of aflatoxin B_1 and G_1 in Neurospora crassa. Mutation Research 37:27-32, 1976.

Ong, T.: Aflatoxin mutagenesis in Neurospora crassa. Proceedings of the 5th International Symposium on Animal, Plant and Microbial Toxins, in press.

Callen, D. F. and Ong, T.: Effects of the epoxidehydrase inhibitor, 1,1,1-trichloropropane-2,3-oxide on the genetic activity of aflatoxin B_1 metabolites in in vitro activation test systems. Mutation Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60041-02 LEM

PERIOD COVERED
July 1, 1976 to May 31, 1977

TITLE OF PROJECT (80 characters or less)
Synergistic Effects of Radiation and Chemical Mutagens on Spermatogonia in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LEM NIEHS
	E. Soares	Senior Staff Fellow	LEM NIEHS
Other:	R. Sorg	Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations using known chemical mutagens have failed to show induction of dominant lethal mutations in spermatogonial stem cells of mice. Studies with irradiation have shown that delivering a dose in two fractions increased the mutation rate, thus implying that the first fraction caused the stem cells to enter a more sensitive state by the time of the second fraction. We are investigating whether pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemical treatment following the irradiation. Comparisons are being made between groups of animals receiving the combined treatment, radiation alone or only chemical treatment for induction of dominant lethals and chromosome aberrations.

PROJECT DESCRIPTION

METHODS EMPLOYED: Adult male mice were irradiated with 300 R of gamma rays. Twenty-four hours after irradiation, they were given a dose of a known potent chemical mutagen (TEM) and thereafter mated in dominant lethal dissection studies. The animals will go through a sterile period caused by the irradiation after which the germ cells tested will correspond to cells in the spermatogonial stem cell stage at the time of treatment. Results are compared with those of animals receiving only irradiation or only chemical treatment as well as with those of a control group in order to determine any increase in the rate of dominant lethal mutations. Samples of males from all groups were examined cytologically to determine the rate of induced chromosome aberrations.

MAJOR FINDINGS AND PROPOSED COURSE: The combined treatment has a strong effect on post-meiotic stages leading to an earlier onset of the sterile period. Comparisons between the groups indicated that there was no discernible induction of dominant lethals or chromosome aberrations in spermatogonia by the chemical alone. No significant differences between the irradiation group and the group receiving both irradiation and chemical were detected in stem cell stages. Further studies will investigate the underlying mechanisms of variation in sensitivity in stem cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mutations occurring in spermatogonial stem cells are of major concern since they not only will deliver large numbers of germ cells carrying identical mutations but will do so for the remainder of the individual's fertile life. Knowledge of the degree of sensitivity of stem cells to mutation induction is pre-requisite to studies intending to estimate the risks from mutagenic agents.

PUBLICATIONS

Sheridan, William, Soares, E. R., and Sorg, Ruth: The combined effects of irradiation and a chemical mutagen (TEM). Annual Meeting of the Association of Industrial Hygienists, May 22-27, 1977, New Orleans, Louisiana (abstract).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60042-02 LEM

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Recessive Lethal Mutations Induced in Mice by Chemical Mutagens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LEM NIEHS
Other:	E. Soares	Senior Staff Fellow	LEM NIEHS
	M. Segall	Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Mutagenesis

SECTION

Mammalian Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.4	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Studies of the induction of recessive lethal mutations in mice by chemical mutagens are being conducted. Backcross progeny between F_1 offspring to chemical mutagen treated parents and their F_2 daughters are used to detect the presence of recessive lethals. An increase in fetal mortality is used as indicator of the lethal. The induction of recessive lethal mutations by irradiation has previously been demonstrated in the mouse. No adequate study of this class of mutation has been conducted in mice following treatment with chemical mutagens. It is our purpose to investigate whether chemical mutagens will induce recessive lethals in mice and the mode of action and long-term effects of such mutations on mammals.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Inbred male mice of the CBA strain were treated with a known chemical mutagen (TEM) and mated to untreated females of the same strain. F_1 male progeny will be collected and individually mated to females of the strain. A series of backcrosses between F_2 females and their sires will be conducted to determine whether there is an increase in fetal mortality indicative of the presence of a recessive lethal.

MAJOR FINDINGS AND PROPOSED COURSE: F_1 animals are presently being weaned and collected. Future extensions of these studies will include investigations of the effects of recessive lethals on early developmental stages in homozygotes, comparisons of heterozygotes with normal homozygotes and attempts at biochemical identification of the loci in question.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is estimated that on the average the human genome contains 5 deleterious recessive genes. Although there are known examples of genes which have an extreme effect in the homozygote also showing an effect in the heterozygote, for the most part little is known about the mode of action or long-term effects on the bearer such genes. Studies are necessary to determine frequencies of induction of this class of genes by mutagenic agents and their consequences to the individual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60043-02 LEM

PERIOD COVERED
July 1, 1976 to November 30, 1976

TITLE OF PROJECT (80 characters or less)

A Pharmacokinetic Study of the Distribution of Triethylenemelamine in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Soares	Senior Staff Fellow	LEM NIEHS
Other:	H. Matthews	Research Chemist	PB NIEHS
	T. Eling	Co-Section Head	PB NIEHS
	M. Anderson	Co-Section Head	PB NIEHS

COOPERATING UNITS (if any)

Pharmacology Branch, Environmental Biometry Branch

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

TEM is a widely used reference chemical mutagen, both as a positive control in routine testing and as the mutagen of choice in the developmental aspects of the mutagenesis programs. The goal of this project is to gain a better understanding of the mode of action of TEM through studies of the pharmacokinetics of this compound. Our results indicate a rapid turnover and elimination of TEM regardless of the placement of the label. However, we have found that with oral administration of TEM, the ethyleneimine portion of the molecule enters the blood in relatively small amounts as compared to i.p. injections.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: TEM (ethyleneimine-1,2^{14}C) or TEM (triazine-2,4,6^{14}C) was administered by gavage or by i.p. injection to male mice. At one and twenty-four hours after injection, males were killed and various tissues were prepared for oxidation for subsequent analysis. Comparisons of levels of radioactivity in the tissues were made within animals, between compounds, between and within routes of administration, and between time periods.

MAJOR FINDINGS AND PROPOSED COURSE: As indicated in the earlier report, the preliminary experiments have been repeated and the initial results confirmed. As of November 1976, this project was terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60044-02 LEM

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical Genetic Variation: Its Measurement and Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Senior Staff Fellow	LEM NIEHS
Other:	D. Smith	Computer Programmer	EBB NIEHS

COOPERATING UNITS (if any)
Environmental Biometry Branch

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	1.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Population geneticists have been aware of the enormous genetic variation at biochemical level in natural populations (including humans) for some ten years. No satisfactory interpretation of the biological significance of the subtle variations has been identified. The objective of this project is to discern association among various biochemical polymorphisms that might indicate their physiological or developmental effects.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Second and third chromosomes were genetically extracted from natural populations of Drosophila melanogaster. These were then genotyped for some 20 enzyme loci and karyotyped.

MAJOR FINDINGS AND PROPOSED COURSE: The results of these studies indicate little non-random association among allozymes in natural population. At face value this is consistent with electrophoretic biochemical polymorphisms being selectively insignificant. There are, however, several qualifications of these analyses which are discussed in the reports. This project has come to an end.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: If genetic variation at various enzyme loci were strongly correlated then this would indicate that this polymorphic enzyme variation is biologically significant, i.e., the viability and/or fecundity of an individual is sensitive to the particular allele of each enzyme which he possesses. This in turn would reflect on our evaluation of newly arising mutants.

PUBLICATIONS

Langley, C. H., Ito, K., and Voelker, R. A.: Linkage disequilibrium in natural populations of Drosophila melanogaster. Seasonal variation. To appear in Genetics.

Langley, C. H.: Non-random associations between allozymes in natural populations of D. melanogaster. In the publication of the proceedings of the symposium "Measurement of Natural Selection," Denmark 1976, accepted for publication by Springer Verlag, NY, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60047-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mutation Induction in Chinese Hamster Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. Huang Senior Staff Fellow LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.4	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Factors influencing the frequency of 6-thioguaninine resistant mutations were examined in Chinese hamster lung cells. The increment in the plating efficiency of treated cells as a function of expression time, spontaneous mutation rate per cell per generation, viability of mutant as well as wild-type cells, and half life of HGPRTase were also evaluated. Continuous diminution in the level of HGPRTase was interpreted as an important factor responsible for the recovery of mutants during the expression time. This cell line was suggested to be suitable for mutagenicity testing in the induction of mutation at the HGPRT locus.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The methodologies of culturing mammalian cells, drug-resistant selective systems, enzyme assays, protein determination, and population genetics were used.

MAJOR FINDINGS AND PROPOSED COURSE: Over a range of doses, a longer expression time is required for mutant cells from a more damaged population to reach their maximum frequency. Diminution in the level of HGPRTase was considered to be a major factor for the recovery of mutants in the selective medium. None of a large number of mutants sampled from those isolated had HGPRT activity. It is suggested that this cell line is suitable for mutagenicity testing in the induction of mutation in the screening of environmental mutagens and carcinogens. We propose to characterize this system further with regard to other agents and the nature of the genetic lesions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The induction of 8-azaguanine and 6-thioguanine resistant mutants in cultured mammalian cells is now well established, and there are indications that the system may prove useful as a screening test for environmental mutagens. There are, however, variations in the response of the system. Any of these variations, if not properly overcome, will influence the estimation of mutation frequencies, and at worse could lead to a false negative determination of the mutagenicity of an agent. It is our objective to identify and elucidate the sources of variation in this genetic testing system.

PUBLICATIONS

Huang, S. L.: Dilution of hypoxanthineguanine phosphorybosyl transferase and other factors affecting the frequency of 6-thioguanine resistance in Chinese hamster lung cells. Mutation Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60048-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetic Effects of Crocidolite Asbestos in Chinese Hamster Lung Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Huang	Senior Staff Fellow	LEM NIEHS
Other:	D. Saggioro	Visiting Fellow	LEM NIEHS
	H. Michelmann	Visiting Fellow	LEM NIEHS
	H. Malling	Chief	LEM NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The genetic effects of asbestos on cultured cells were studied in order to correlate them to asbestos carcinogenicity. This is achieved by the investigation of cytotoxicity effects, chromosomal aberrations, phagocytosis and induced gene mutations by the crystals. The occurrence of pleural and peritoneal mesotheliomas and carcinomas in the gastro-intestinal tract is linked with the inhalation of asbestos fibres. Experimentally, mesotheliomata have been induced by the intrapleural inoculation of fibres into rats. Experiments also showed that very large amounts of asbestos dust could be taken up by individual lung macrophages and remain in macrophage-converted fibroblasts. These findings suggest that the incorporation of the fibres disturbs processes at the cellular level.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The methodologies of culturing mammalian cells, cytological preparations, electron microscopic slide preparations and induction of 6-thioguanine resistance were used.

MAJOR FINDINGS AND PROPOSED COURSE: Chinese hamster lung cells cultured in the presence of crocidolite asbestos displayed inhibition of cell growth. Cell death was directly associated with the phagocytosis of larger fibres. Water soluble components of asbestos did not appear to inhibit cell growth. Chromosomal aberrations were induced and were confined mainly to structural aberrations - breaks and fragments. Electron microscopic preparation indicated that asbestoses were readily contained in phagosomes. Phagocytosed asbestos did not appear to induce gene mutation at the hypoxanthine-guanine phosphoribosyl transferase locus. The evidence of the induction of gene mutation was rather weak. We propose to continue our study with other species of asbestos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The importance of asbestos dust and submicroscopic materials has become widely recognized. These submicroscopic particles might be an important dimension in the carcinogenesis by asbestos. In contrast with the abundant literatures on the carcinogenicity of asbestos in both men and animals, there are virtually no reported studies on its mutagenicity. The study of the genetic effects of asbestos will help explicate the relationship between mutagenesis and carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60049-01 LEM

PERIOD COVERED
September 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of Enzyme Induction in the Metabolism of Mutagens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Section Head	LEM NIEHS
Other:	R. Chhabra	Senior Staff Fellow	PB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project is designed to measure the effects of Arochlor-1254 induction of Sprague-Dawley and Fisher 344 rats. A 9000 x g post mitochondrial supernatant of livers from these rats is used to activate benzo(a)pyrene (BP), dimethylnitrosamine (DMN), and 2-amino anthracene (2AA) to mutagens in the Ames Salmonella plate test. Simultaneously, BP hydroxylase and DMN demethylase activities are being measured, as well as Cytochrome P448/450, aminopyrine demethylase, and analine hydroxylase. An attempt is being made to see if mutagenicity is an accurate correlate of microsomal enzyme activity.

PHS-6040
(Rev. 10-76)

MAJOR FINDINGS AND PROPOSED COURSE: This project has been recently initiated and definitive results are not yet available. The future plans include completing the study of the effects of Arochlor 1254. We expect to expand the study to study other inducers such as phenobarbital and 3-methylcholanthrene, alone, and in combination to see how their effects compare with the effects of Arochlor-1254.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will demonstrate whether mutagenicity in the Salmonella plate test can be used as reliable correlate of liver enzyme activity. Also, it will provide us with data on strain differences in mutagen metabolism and responses to Arochlor 1254 induction.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60050-01 LEM

PERIOD COVERED
November 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Development of an Intrasanguinous Host-Mediated Assay Using Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Frezza	Visiting Fellow	LEM NIEHS
Other:	E. Zeiger	Section Head	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

To extend the intrasanguinous host-mediated assay system to measure the organ distribution of mutagens in mice using Saccharomyces cerevisiae and Schizosaccharomyces pombe as indicator organisms. The distribution of the yeast in the different organs will be measured as will the mutagenicity of various chemicals requiring metabolism to their active forms. The distribution of mutagen in the various organs will be determined and related to other in vivo effects of the chemical.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Yeast suspensions (0.2 ml of 3 x 10^8 cells/ml) are injected into the tail vein of mice and the chemical to be tested is given orally. The yeast is retained in the liver, lungs, kidneys, and testes. At selected times, the mice are sacrificed and the organs removed and homogenized in a tissue grinder to free the yeast from the tissue. Mutation and mitotic gene conversion and recombination can then be determined using routine procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Mice of strain CD-1 appear to be the most resistant to the stress of the procedure. The distribution of the cells in the various organs is time-dependent. Right after the injection, most of the cells are found in the lungs; after 10 minutes an increasing number of cells are found in the liver and the spleen, and finally up to 40% of the total injected number of cells can be recovered from the liver. The mouse does not appear to have any pathological reaction toward the presence of the yeast in the body. The recovery of cells from liver, kidneys, and lungs is sufficient to perform mutagenesis tests with Saccharomyces cerevisiae strains as D_4 and D_5 up to 18 hours after the injection. We will begin testing specific chemicals of different chemical classes whose organ distribution is known in order to determine the sensitivity of the system. In addition the results will be compared to results in an in vitro metabolic activation system to see if the types of genetic activity obtained varies between the in vivo and in vitro systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been well documented that different organs are capable of metabolizing different chemicals at different rates. Using this system the organ specific metabolism of mutagens can be determined in vivo and possibly have predictive value for other in vivo toxic effects. Also, it is hoped that this procedure would be useful for routine assay of potential mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60051-01 LEM

PERIOD COVERED

November 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Use of Salmonella in an Intrasanguinous Host-Mediated Assay for Measuring Mutagenic Chemicals in Mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Section Head	LEM NIEHS
Other:	J. Bernhein	Microbiologist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Mutagenesis

SECTION

Microbial Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Salmonella typhimurium strains are being used in an intrasanguinous host-mediated assay in mice to see if this in vivo system is capable of detecting a wide variety of chemical mutagens which require metabolic activation for their genetic activity. The effective dose ranges of the chemicals will be determined and compared with results obtained using in vitro activation techniques.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: This project has been recently initiated and chemical testing has not yet begun. Preliminary studies have shown that if 10^9 cells are injected, 10-30% recovery can be obtained in one hour. Chemicals belonging to different classes of known mutagens - polycyclic aromatic hydrocarbons, aromatic amines, nitro and nitroso compounds, mycotoxins, and chlorinated hydrocarbons, which have been previously tested using an *in vitro* metabolic activation system, will be tested in this *in vivo* system. Additionally, a number of chemical carcinogens which are not mutagenic *in vitro* will be tested to see if the *in vivo* system can detect them.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A large number of chemical mutagens require mammalian activation in order to be mutagenic for bacteria. The primary way of looking at this metabolism at present is by the use of liver homogenates *in vitro*. However, there are a number of chemicals which do not appear to be activated *in vitro* but are activated *in vivo*. Also, there is concern that *in vitro* metabolism does not always mimic *in vivo* metabolism, and results obtained *in vitro* may not give a true indication of what is happening in the intact animal. This program is designed to see whether the intrasanguinous host-mediated assay can be used for routine mutagen testing and to examine the correlation between *in vitro* and *in vivo* results.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60052-01 LEM

PERIOD COVERED
October 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mutagenesis Testing Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Valcovic	Geneticist	LEM	NIEHS
Other:	E. Zeiger	Section Head	LEM	NIEHS
	T. Ong	Geneticist	LEM	NIEHS
	J. McKinney	Section Head	EBCB	NIEHS
	D. Hoel	Branch Chief	BB	NIEHS

COOPERATING UNITS (if any)
Environmental Biology & Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The primary objective of this effort is to develop a program which will have the ultimate capability of testing 1000 chemicals per year using microbial systems, and as-yet-undetermined smaller numbers of chemicals in higher in vitro and in vivo test systems. With this level of testing, it will be necessary to develop the ability for chemical management and analyses of compounds tested. A computerized system for data handling and statistical analysis will also be developed.

Substances which are mutagenic in the microbial tests will be tested further using higher systems which serve to affirm or refute the microbial data and more precisely define the types of mutagenic events induced.

PHS-6040
(Rev. 10-76)

MAJOR FINDINGS AND PROPOSED COURSE: This program is a new effort and will be conducted utilizing the contract mechanism. The staff to develop these contracts is currently being recruited. It is anticipated that the chemical repository will be operational and will develop an efficient inventory and chemical management system. Tier-1 microbial testing will begin with 150 chemicals in early FY78. Test systems for Tiers 2 and 3 will be selected. Additionally, an on-line computerized data handling system will be developed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There are literally thousands of chemicals in use and the number is ever increasing. The majority of these substances has not been evaluated for the potential to induced heritable genetic alterations; and, hence, the possible risk to future human generations is unknown. This program will develop an efficient coordinated system for mutagenesis testing and should serve as a model system. The data obtained from this program will be useful for decision making on the safety of various classes of chemicals to which man is exposed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60053-01 LEM

PERIOD COVERED
August 15, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Host-Mediated Assay Using Neurospora crassa as Indicator Organism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Whong	Visiting Fellow	LEM NIEHS
Other:	T. Ong	Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A large number of chemicals are not mutagenic unless they are converted enzymatically to active metabolites. The host-mediated assay is a useful and versatile tool for determining whether chemical agents are being converted to mutagenic metabolites by the host animals. This assay has been well established and employed extensively with indicator organisms such as bacteria and yeast. In Neurospora crassa, the host-mediated system has also been developed. However, no extensive studies have been carried out. It is, therefore, necessary to extend the host-mediated assay system using Neurospora as an indicator organism to different animal species, different organs and different procarcinogens.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: For increasing sensitivity to chemicals, an excision-repair deficient strain of N. crassa (H-59) was employed as an indicator organism. 0.2 ml of the conidial suspension (2 x 10^9 conidia/ml for rats, 10^9 conidia/ml for mice) was injected into the tail vein of each animal. The chemical solution was administered into animals by intramuscular injection. The animals were sacrificed after 16 hours of treatment. Conidia were harvested from different organs of the host. The ad-3 forward-mutation frequency of N. crassa was measured by the direct method of F. J. de Serres.

MAJOR FINDINGS AND PROPOSED COURSE: In mice, dimethylnitrosamine (DMNA) appears to be converted to highly mutagenic metabolites in liver. This is in agreement with that of Malling's finding. A significant increase in mutation frequency was also found among the conidia recovered from the lung of DMNA-treated mice. In the liver of rats, DMNA is also converted to highly mutagenic in Neurospora. Studies will be extended to (1) different animal species, (2) other organs (kidney, lung and testis) and (3) other procarcinogens from different chemical classes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the host-mediated assay can provide important information in regard to metabolic activation of chemical carcinogens in vivo, which, in turn, can provide more reliable information on the potential mutagenic and carcinogenic hazard of chemical agents to man. The studies on the activation of chemicals into mutagenic metabolites by various organs can also provide information on the correlation between the mutagenicity and carcinogenicity of environmental agents, and on the mechanism of carcinogenesis and mutagenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60054-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Chemically Induced Mutations at Biochemical Loci in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. R. Soares Senior Staff Fellow LEM NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	0.5	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Although in theory alkylating agents such as TEM should be capable of inducing gene mutations in mammals, only a relatively small amount of data are available supporting this hypothesis. The objective of this study was to test the effectiveness of a chemical mutagen in inducing mutations at a number of different enzyme loci in the mouse. Strain DBA/2J male mice were exposed to triethylenemelimine (TEM) by i.p. injection. Control males were injected with the carrier (HBSS). Males were mated to C57BL/6J females. Kidneys and blood were obtained from all F_1 offspring and said tissues were analyzed by starch gel electrophoresis for the occurrence of mutations at the various enzyme loci. A number of putative mutants have been identified in this experiment and progeny testing is underway to confirm these mutations.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: DBA/2J male mice were treated by i.p. injection with TEM. Control males were treated with HBSS. All males were mated to C57BL/6J females. Tissues (kidneys and blood) were sampled from all F_1 offspring of the above matings. Using standard starch gel electrophoretic techniques, all samples were electrophoresed and the gels analyzed for the occurrence of aberrant zymogram patterns. Such aberrant patterns were considered indicative of putitive mutations. Animals bearing putitive mutations will be studied by standard progeny testing techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Several putitive mutations have been recovered at the following loci, Es-1, PGM-1, LDH, Es-3. Animals bearing the suspect mutations are presently undergoing progeny testing. This experiment will be completed shortly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A knowledge and understanding of the types of genetic lesions induced in mammals by chemical mutagen and the relationship between the genetic effects of known mutagens and environmental agents which are potentially mutagenic is vital to our understanding of the risk posed to humans by such environmental agents. This study will aid in our understanding these genetic effects and will provide basic information regarding the mechanism of action of a known potent chemical mutagen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60055-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Inactivation and Mutagenesis in Doubly Repair-Deficient Strains of Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Inoue	Visiting Fellow	LEM	NIEHS
	R. Harvey	Geneticist	LEM	NIEHS
Other:	D. Callen	Visiting Fellow	LEM	NIEHS
	F. de Serres	Associate Director for Genetics	OD	NIEHS

COOPERATING UNITS (if any)
Miles Laboratories, Elkhart, IN

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A number of loci which control radiation sensitivity in Neurospora crassa have been identified. The DNA repair processes have been characterized to some extent on the bases of responses of mutant strains to radiations and chemical agents. This project is an analysis of the interactions of mutant alleles that individually confer radiation sensitivity with regard to their effects on radiation-induced inactivation and forward-mutation induction. The lethal and mutagenic effects of UV and x-ray irradiation in these double-mutant strains are interpreted in terms of the repair systems in Neurospora.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: We have constructed several homokaryotic double-mutant strains of N. crassa which bear in various pair-wise combinations the radiation sensitive mutant alleles: upr-1, uvs-2, uvs-3, and uvs-6. Inactivation and forward-mutation induction at two loci by UV and x-ray irradiation have been determined by use of the ad-3 system.

MAJOR FINDINGS AND PROPOSED COURSE: Our data for the double-mutant strain bearing the two excision repair deficient alleles, upr-1, and uvs-2, have shown that these interact epistatically with respect to the lethal and mutagenic effects of UV irradiation. However, they show an additive type of interaction for both inactivation and mutagenesis by ionizing irradiation. This additive interaction indicates that, in response to the lesions induced by ionizing irradiation, the gene products from the wild type upr-1(+) and uvs-2(+) alleles are involved in repair processes that are to some extent independent.

The uvs-2, uvs-6 combination of mutant alleles results in an increased sensitivity to inactivation by both UV and x-ray irradiation. We have determined that the interaction of the uvs-2 and uvs-6 alleles results in a reduction in the frequencies of ad-3 mutants (forward-mutation frequency per survivor, relative to the frequencies obtained for the more sensitive of the two single-mutant parental strains) for both UV and x-ray irradiation.

The combination of the uvs-3 and uvs-6 mutant alleles has been shown by tetrad analysis to be inviable.

Additional double-mutant strains are being tested for their sensitivities to inactivation and forward mutation induction by irradiations. The qualitative characterization of ad-3 forward mutants induced in these doubly repair deficient strains is currently being conducted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many genetic factors are involved in the responses of an organism to pre-lethal and pre-mutagenic lesions. Earlier studies have shown that the presence of repair deficiencies in test organisms alters the effects of lethal and mutagenic agents both quantitatively and qualitatively. In order to understand the complex biological processes involved in DNA repair and mutagenesis, it is important to consider the interactions of these gene products. The types of interactions of multiple repair deficiencies within the cell indicate the role and significance of particular gene products in the various repair pathways that are available in response to lesions in the DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60056-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Forward-Mutation Induction by Tritiated Water in a Two-Component Heterokaryon of Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. C. Harvey	Geneticist	LEM NIEHS
	F. J. de Serres	Associate Director	OD NIEHS
	R. B. Cumming		Oak Ridge Nat'l Laboratory

COOPERATING UNITS (if any)

Oak Ridge National Laboratory, Oak Ridge, TN
Miles Laboratory, Elkhart, IN

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The ad-3 test system in a two-component heterokaryotic strain of Neurospora crassa is being used in an analysis of inactivation and forward-mutation induction by tritiated water. Genetic analysis of induced mutants will make possible a qualitative as well as quantitative characterization of tritiated water mutagenicity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Only pilot experiments have been completed to date. However, preliminary data tend to indicate that the relative biological efficiency of tritiated water for inactivation is rather high, and that the relative efficiency for ad-3 forward-mutation induction may approximate unity. Additional experiments will be conducted to determine the relative biological efficiencies of tritiated water for the induction of a variety of genetic lesions within the same test organism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tritium, in the form of tritiated water, is one of the primary pollutants resulting from nuclear power production. It is difficult and expensive to contain. Since it is unimpeded by biological barriers, tritiated water rapidly achieves an equilibrium concentration in living organisms that is equal to that in the environment. The biological effectiveness of the tritium β-particle is the primary consideration in hazard evaluation. This β-particle is emitted at a very low energy by tritium decay, the average track length being only 0.9 μ in biological materials. As a consequence of its very short track length, the energy is expended in a very localized region. The tritium decay product is, therefore, a weakly penetrating but high linear energy transfer radiation. These characteristics make tritiated water an environmental mutagen of great practical and theoretical importance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60057-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparative Restriction Endonuclease and Electron Microscopic Analyses of the Mitochondrial DNAs (mt-DNA) of Different Species of Drosophila

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Senior Staff Fellow	LEM NIEHS
	D. Shah	Visiting Fellow	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

We have done preliminary characterization of mitochondrial DNAs isolated from three species of Drosophila. Electron microscope heteroduplex and restriction endonuclease analyses have revealed that these mtDNAs have undergone a significant evolutionary divergence in their nucleotide sequences. We are constructing specific cleavage maps of these mtDNAs using various restriction endonucleases. Restriction endonucleases recognize specific base sequences within a DNA molecule and have proven useful in mapping genes and detecting single base-pair substitutions. Utilizing this information, our objectives are to understand (1) organization of genes as related to function, (2) how the gene arrangements change, (3) the rates of evolution of small DNAs, and (4) heterogeneity of intraspecific mtDNA.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: CsCl-ethidium bromide centrifugation, restriction endonuclease analysis using gel electrophoresis, electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: MtDNAs of D. melanogaster and D. simulans have similar molecular weights (11.9×10^6) while D. virilis mtDNA has a molecular weight of 10.0×10^6 as determined by electron microscopy. Hind III restriction endonuclease cleavage patterns of D. melanogaster and D. simulans are identical and easily distinguishable from that of D. virilis. The smaller size of D. virilis also has been confirmed by electron microscope heteroduplex analysis. While Hind III patterns of D. melanogaster and D. simulans mtDNAs are identical, Eco RI and Hae III patterns are not, suggesting single base-pair substitutions in these DNAs. This work is already in progress and will be completed soon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies would help understand the sequence complexity of mtDNAs and would eventually be extended to specific nuclear genes isolated using restriction enzymes and amplified by DNA cloning.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60058-01 LEM

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Amount and Effects of Null Allozymic Variation in Natural Populations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Voelker	Senior Staff Fellow	LEM NIEHS
Other:	C. Langley	Senior Staff Fellow	LEM NIEHS
	A. Leigh-Brown	Visiting Fellow	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.7	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Virtually nothing is known about the frequency of "null" allozyme alleles (alleles which code for the production of no enzyme activity) in natural populations or their importance to the well-being of the individuals which carry such alleles. This study seeks to answer these questions for several natural populations of Drosophila melanogaster.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Extraction of chromosomes from widely separated natural populations utilizes dominantly marked balancer chromosomes. Electrophoretic assays utilize gel electrophoresis preparation and staining techniques. Cytological analyses involve examination and photography of salivary gland chromosome preparations. Immunologic assays involve preparations of antibodies to purified wild type enzymes and detection of cross-reacting materials.

MAJOR FINDINGS AND PROPOSED COURSE: The study is at a stage where nearly all materials to be used in the screen for "nulls" have been synthesized. This involved the identification of as many electrophoretically assayable enzyme loci as possible located on chromosomes I, II and III of Drosophila melanogaster. In addition to 22 previously described loci, we have mapped and/or discovered electrophoretic variants at 9 new loci, these being adenylate kinase (2 loci), hexokinase AB, glutamatepyruvate transaminase, dipeptidase-A, phospho-glucose isomerase, mitochondrial malate dehydrogenase, aldolase and triose phosphate isomerase. These loci are being or have been genetically and cytogenetically mapped.

Since "null" alleles can be detected by their failure to show a heterozygous banding pattern with two mobility variants, it is necessary to construct chromosomes carrying alternative alleles which can be used as "testers." Much of our effort has been devoted to detecting mobility variants at the enzyme loci and recombining these together into the smallest possible number of "tester" stocks to minimize the effort to screen for nulls.

The next six to eight months will be spent screening flies collected from natural populations for "nulls." Following that, the genetic, cytogenetic and immunogenetic analyses will be carried out. The amount of time required for these analyses will be largely determined by the yield of "nulls" from the screen.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information obtained from this study is vital to an understanding of the genetic variation which exists in natural populations, both as regards to amount and type (i.e., base substitutions, deletions). Once the amount and types of "natural" variation is known, it will be possible to assess the effects attributable to new environmental mutagens. Moreover, the biological effect of homozygosity for "null" alleles, based on limited evidence, presents a conflicting picture; in humans such effects are thought to be severely detrimental, while in Drosophila the effects appear to be less drastic. By detecting a large number of "nulls" at many loci, it should be possible to draw more accurate conclusions for Drosophila and ultimately for mammals, including man.

PUBLICATIONS

Voelker, R. A., and Langley, C. H. (1977) Cytological localization of Roi (rough eye). Drosophila Information Service 52.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60059-01 LEM

PERIOD COVERED
January 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Chromosomal Aberrations on Embryonic Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LEM NIEHS
Other:	H. Michelmann	Visiting Fellow	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Mammalian Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The project is designed to study the effects of chromosomal aberrations on fetal mortality and development. Comparisons will be made between in vivo effects and effects on mouse embryos grown in culture. Chemical mutagens are known to be capable of inducing chromosomal aberrations such as translocations which may be transmitted to the next generation. Increased rates of fetal death are usually observed among offspring to translocation heterozygotes. It is our purpose to investigate the rates of transmission of such aberrations, and the processes of fetal mortality by direct observation of the development of embryos.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Known translocation bearing mice are mated and the F_1 sons studied to determine the frequency of translocation heterozygotes. In other matings the females are killed shortly after fertilization and two cell embryos are retrieved from the oviducts and established in culture. Stage of development arrest is determined.

MAJOR FINDINGS AND PROPOSED COURSE: F_1 males are currently being analyzed to determine whether they are translocation heterozygotes. Cytogenetic analysis will be conducted in attempts to correlate the nature of the aberration and the effect on embryonic development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chromosome aberrations in man are known to cause disturbances in development of embryos leading to death of the embryo or to abnormal offspring. An understanding of the fate and effects of chromosome aberrations which may be caused by environmental agents is necessary for evaluation of the possible risks such induced aberrations may imply for man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60060-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Use of Enzyme Heat Denaturation for Detection of Mutations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Malling	Chief	LEM NIEHS
Other:	J. Burkhart	Chemist	LEM NIEHS
	C.-Y. Lee	Senior Staff Fellow	LEM NIEHS
	B. Pegoraro	Visiting Fellow	LEM NIEHS

COOPERATING UNITS (if any)
Biometry Branch, Laboratory of Environmental Biology and Chemistry

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.5	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k-

The objective of this project is to develop a system to detect induced mutations in mice by using high speed rate analyzers to monitor the thermal stability of enzymes. Pilot experiments are in progress to determine the biochemical conditions necessary to screen crude homogenates of mouse tissues for thermal stability mutants of 20 enzymes. Preliminary results indicate that enzyme heat denaturation is closely correlated to pH, ionic strength, and endogenous concentrations of substrates and cofactors. In addition naturally occurring variants of isocitrate dehydrogenase and phosphoglucose isomerase have been found to be detectable with heat stability techniques.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The thermal stabilities of purified enzymes and enzymes in crudely homogenized tissues are being comparatively studied to understand the relationship that exists between thermal stability of an enzyme based upon structure alone and thermal stability of the enzyme as a function of physiological environment and method of preparation. The knowledge of these relationships will be applied to defining a limited number of conditions that are feasible for use in a large scale experiment.

The major premise of this methodology is that for a given inbred mouse population, there exists a normal relative thermal stability for a given set of experimental conditions for each enzyme. Data on control and mutagen-treated mice could be accumulated with the Miniature Centrifugal Fast Analyzer and integral PDP11 Computer, then transferred to the Institute's computer. In-depth statistical analysis could be accomplished through the Statistical Analysis System in Washington, D.C.

MAJOR FINDINGS AND PROPOSED COURSE: Conditions such as pH, ionic strength and substrate concentration have been found to alter thermal stability of mouse kidney enzymes at constant time and temperature in both crudely homogenized and purified enzymes. Homogenates of naturally occurring electrophoretic variants that are indistinguishable by thermal stability at neutral pH (7.5) may be resolved at a lower pH (6.0); the resolution can be improved by variation of ionic strength. Model studies to demonstrate these effects are being completed and 20 enzymes are being tested to establish the best conditions for subsequent studies. Computer programs to complement this methodology are being developed. Relative thermal stability will be determined for individual mice within and between litters for a selected number of kidney enzymes. The system will then be tested on a mutagen-treated population to evaluate its capacity to quantitate the frequency of mutation induction. Development of an analytical system based upon the erythrocyte will be concurrent.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Development of this system is important because it could provide a rapid means to screen mammalian populations for mutational events at many loci. In addition, measurements of enzyme thermal stability can potentially distinguish regulatory variations as changes in specific activity and structural variation as changes in relative thermal stability.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60061-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical Characterizations of Genetic Variants and Isozymes in Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.-Y. Lee	Senior Staff Fellow	LEM NIEHS
Other:	B. Pegoraro	Visiting Fellow	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study is to conduct a model study to differentiate the enzyme variants in mice by simple biochemical assays. Therefore, several variants and isozymes in two inbred strains of mice were purified, and experimental conditions to differentiate these variants or isozymes were established. These include thermostability, urea and iodoacetate inactivation, and kinetic studies. From these studies, the electrophoretic variants of 3-phosphoglycerate kinase-2 and the isozymes of Isocitrate dehydrogenase could be differentiated by their relative thermostability. From this model study we think that relative thermostability of enzyme variants could be a useful tool for the detection of mutant enzymes in mutagen-treated mouse population.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: For the purification of the enzymes, a two-step procedure has been employed, involving affinity chromatography as a major step in the purification. The purified enzymes have been characterized biochemically and the kinetics of inactivation under several experimental conditions have been studied.

MAJOR FINDINGS AND PROPOSED COURSE: Two isozymes of phosphoglycerate kinase and isocitrate dehydrogenase have been characterized by kinetic studies, thermal and urea denaturation and iodoacetate inactivation. The conditions to find maximum difference by thermal and urea denaturation were established. Two genetic variants of cytoplasmic isocitrate dehydrogenase and 3-phosphoglycerate kinase-2 were also compared biochemically in details. Differential thermal stability was also observed for each pair of enzyme variants under certain temperatures and pH's. Further studies are being conducted to find if other naturally occurring enzyme variants (electrophoretic variants) in mice also show thermal stability difference under some experimental conditions. We hope that we are able to generalize these experimental conditions so that one is able to utilize them for the practical screening of mutant enzymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: This project is important in the development of a rapid and simple biochemical assay to detect the mutant enzymes in mutagen-treated animal populations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60062-01 LEM

PERIOD COVERED
January 15, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular Characterization of Isozymes and Mutant Enzymes from Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Li	Research Geneticist	LEM NIEHS
Other:	R. Chen	Visiting Scientist	LEM NIEHS
	C-Y. Lee	Senior Staff Fellow	LEM NIEHS
	B. Hindenach	Chemist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Various isozymes and mutant enzymes such as phosphoglycerate kinase, isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase from mouse have been purified. These proteins will be characterized by comparison of their peptide maps and determination of their amino acid sequence differences.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Various dehydrogenases are purified from mouse by AMP-sepharose affinity column. Tryptic peptides of these proteins will be analyzed on thin-layer and paper by chromatography and electrophoresis. Some of peptides will be eluded, and then amino acid composition and sequences will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: Various isozymes and mutant enzymes, namely, phosphoglycerate kinase, isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase from mouse have been purified, and their amino acid substitutions will be characterized.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Various isozymes and mutant enzymes in mouse are detected by difference in electrophoretic mobility and/or kinetic parameters in a biochemical specific locus mutation system. Molecular characterization of these mutant proteins will elucidate the nature of genetic mutations in mammals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60063-01 LEM

PERIOD COVERED
January 15, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Complete Amino Acid Sequence Determination of Sperm-Specific Lactate Dehydrogenase from Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Marciniszyn	Senior Staff Fellow	LEM NIEHS
Other:	Y-C. Pan	Visiting Fellow	LEM NIEHS
	C-Y. Lee	Senior Staff Fellow	LEM NIEHS
	S. Li	Research Geneticist	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purification procedure for sperm-specific lactate dehydrogenase from mouse has been developed. Large quantities of the enzyme are being purified. Tryptic peptides and CNBr fragments of this protein will be separated and purified. Amino acid sequences of pure peptides will be determined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Sperm-specific lactate dehydrogenase from mouse are purified by AMP-sepharose column. The protein will be cleaved into peptides with trypsin and CNBr, and these peptides will be purified by gel filtration and ion-exchange chromatography. Amino acid sequences of pure peptides will be determined by automatic Edman degradation on Beckman sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: Sperm-specific lactate dehydrogenase from mouse has been purified, and the complete primary structure will be determined. Various submolecular fragments of this enzyme will also be used to purify monospecific antibodies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The amino acid sequence differences among different isozymes of lactate dehydrogenases will be correlated with their antigenic determinants. This information is essential for mutation monitoring program using monospecific antibodies against this enzyme.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60064-01 LEM

PERIOD COVERED
January 15, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Primary Structure Characterization of Muscular Lactate Dehydrogenase from Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Li	Research Geneticist	LEM NIEHS
Other:	S-M. Chang	Visiting Fellow	LEM NIEHS
	S. Huang	Visiting Fellow	LEM NIEHS
	C-Y. Lee	Senior Staff Fellow	LEM NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline)

Large quantity of muscular lactate dehydrogenase from mouse has been purified. Tryptic peptides and CNBr fragments of this protein will be separated, and the purified peptides will be determined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Muscular lactate dehydrogenase from mouse is purified by affinity chromatography. Peptides of this protein derived from enzymatic and chemical cleavage will be purified by gel filtration and ion-exchange chromatography. Amino acid sequences of these peptides will be determined by automatic Edman degradation.

MAJOR FINDINGS AND PROPOSED COURSE: Large quantity (1.5 g) of muscular lactate dehydrogenase from mouse has been purified, and the primary structure of this enzyme based one peptide sequences will be compared with that of sperm-specific lactate dehydrogenase from mouse.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Amino acid sequence differences among different isozymes of lactate dehydrogenases from mouse will be correlated with their antigenic properties. This is a part of the program to evaluate the molecular events which result in mutations in mammals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60065-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Non-Human Primates in Mutagenicity Testing: Host-Mediated Assay

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. V. Malling	Laboratory Chief	LEM NIEHS
Other:	D. B. Feldman	Veterinarian	EBCB NIEHS
	G. Ficsor	Consultant	Western Michigan Univ.

COOPERATING UNITS (if any)
Division of Laboratory of Animal Resources, Duke University

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Rhesus monkey hosts were used in intraperitoneal host-mediated assay (HMA) of mitomycin C with E. coli K-12, 343/113 cells enclosed in dialyzing tube diffusion bags. Intrasanguneous HMA or blood-mediated assay failed due to low survival of indicator cells. The animals used were also evaluated cytogenetically for chromosomal aberrations in somatic and germinal tissues. MC mutagenicity was detected in each.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: For the intrasanguineous HMA inject i.v. approximately 10^9 E. coli per ml blood. Inject animals i.m. with MC in saline or with saline. Recover cells from liver or testes. For in vitro blood assay incubate 10^9 cells/ml blood with or without MC for 30 minutes. Recover cells. For intraperitoneal HMA enclose 3-5 ml of 10^9 cells/ml Nutrient Broth in dialyzing tubing and implant in intraperitoneal cavity. Inject with MC. Recover cells 3 hours later. For all methods the recovered cells were plated on selective media to determine mutation frequency to arginine and galactose prototrophy. Appropriate in vitro experiments with E. coli and MC were performed.

MAJOR FINDINGS AND PROPOSED COURSE: In the Rhesus monkey intrasanguineous HMA and blood-mediated assay is impossible due to the immediate and strong immune response of the host against E. coli. The number of cells that could be recovered from either liver, blood or testicular tissues was so low after 30 minutes exposure that determination of mutation frequencies was not possible. This difficulty was overcome when cells were enclosed in diffusion bags made from dialyzing tubing (cut off of 6000-8000 MW). In the HMA significant increase in mutation frequency were observed at 0.5 mg/kg b.w. (lower doses were not tested). Mutation frequencies were higher at 1.5 mg/kg, but further increase in dose resulted in a slight decrease in mutation frequency. Ten times higher in vivo doses over in vitro doses induced similar mutation frequencies. The same animals used for the HMA have also been used in cytogenetic experiments reported on elsewhere in the annual report. No further experiments are planned since the feasibility of using monkeys in mutagenicity testing with the HMA has been demonstrated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Apes, the Macaques are among man's closest relatives. The chimpanzee is more than 99% related to man on the molecular level, while Macaques follow closely. Therefore, non-human primates should provide the most relevant animal model when the genetic safety of environmental contaminants, drugs, and food additives is evaluated. Due to limitations of animal availability and cost, genetic safety evaluation of chemical substances in monkeys is necessarily limited to special cases when a large body of information is already available from traditional animal tests. The present experiments have established methodology which can be used if the evaluation of a chemical's genetic safety is of great human importance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60066-01 LEM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Non-Human Primates in Mutagenicity Testing: Somatic and Germinal Cytogenetic Tests with Mitomycin C

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. V. Malling	Laboratory Chief	LEM NIEHS
Other:	H. W. Michelmann	Visiting Fellow	LEM NIEHS
	P. Maier	Visiting Fellow	LEM NIEHS
	D. B. Feldman	Veterinarian	EBCB NIEHS
	G. Ficsor	Consultant	Western Michigan Univ.

COOPERATING UNITS (if any)

Division of Laboratory Animal Resources, Duke University

LAB/BRANCH
Laboratory of Environmental Mutagenesis

SECTION
Biochemical and Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Four cytogenetic techniques were compared to detect the clastogenicity of Mitomycin C in somatic or germinal cells of Rhesus monkeys (Macaca mulatta) in vivo: peripheral lymphocyte and bone-marrow cytogenetics, the micronucleus test in bone-marrow, cytogenetic evaluation of primary spermatocytes. Only the lymphocyte test failed to detect clastogenicity probably due to absence of DNA replication in these cells during treatment. The micronucleus test also failed in samples collected by biopsy. The bone marrow test proved to be as sensitive as the micronucleus test and could be performed with biopsy material. The lymphocyte test may also be potentially useful with testing of chemicals that are not dependent on DNA replication for their action. The cytogenetic test of primary spermatocytes was positive only when treatment was applied during the pre-meiotic S-phase. The pre-meiotic S-phase is 17 days before diakinesis-metaphase I.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mitotic metaphase spreads from blood and bone-marrow or meiotic and spermatogonial metaphases from testes biopsy samples were made following standard procedures. Samples from the same animals were collected 14 days before and 1, 2, 14, 16, 17, 18 days and 3 and 5 months after treatment with Mitomycin C. The micronucleus test was also performed with methods developed for rodents by others.

MAJOR FINDINGS AND PROPOSED COURSE: When the sensitivities of the three cytogenetic methods applicable to somatic cells in vivo were compared, we found that the bone-marrow cytogenetic test and the micronucleus test were about equally sensitive, and were also comparable in sensitivity to the host-mediated assay. The lymphocyte cytogenetic test failed to detect chromosome aberrations was attributed to the known dependence of MC-mutagenesis on DNA replication. In a more general testing scheme, which include chemicals or physical agents that are not dependent on DNA replication for mutagenesis, we still recommend the use of lymphocyte cytogenetics since blood can be readily obtained with minor disturbance to the animals or from humans. On the other hand we have reservations about the applicability of the micronucleus test in an experimental situation when it is highly desirable to keep the animals alive following sample taking. When we attempted to analyze bone marrow obtained from live animals the scoring of micronuclei was impossible due to excessive invation by mature erythrocytes from blood. Bone-marrow cytogenetics suffered from none of the short-comings of the other somatic cell based cytogenetic tests we performed.

Previous work in mice by Adler established that MC induces the greatest frequency of exchanges and fragments when it is applied during the premeiotic S-phase. Using this information we determined the timing of premeiotic S-phase by noting the day of treatment prior to meiotic Diakinesis-Metaphase I which showed peak exchange and fragment frequencies. Results from two MC-treated animals showed that premeiotic S-phase is 17 days before treatment. This observation, if confirmed in future experiments, will have applications for both reproductive research and mutagenicity testing.

Work is presently in progress to confirm our conclusion concerning the timing of premeiotic S-phase. We will attempt sister chromatid exchange as an additional method to be used in non-human primates to evaluate the genetic safety of chemicals. Also an arrangement will be sought with cancer research organizations to hold the animals we use for mutagenicity testing for long-range carcinogenic evaluation. The results of mutagenic and carcinogenic studies, when combined, may provide further evidence to the presently emerging correlation between carcinogenicity and mutagenicity in an experimental animal closely related to man.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Molecular and cytogenetic research of the past 25 years revealed that non-human primates and man show greater evolutionary relatedness than was suggested by earlier

morphologic and paleontologic evidence. Therefore, it appears self evident that non-human primates need to be used in research which intends to determine the genetic safety of environmental contaminants, drugs and food additives to man. We believe that with the methods developed for monkeys in this investigation we will be able to provide genetic safety evaluation of chemicals to man more accurately than would be possible with animals less closely related to man.

GEORGIA INSTITUTE OF TECHNOLOGY - ATLANTA, GA 30332
(NIH-NO1-ES-5-2135)

TITLE: Development of a Polygenic Assay for Point Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: John W. Crenshaw, Jr., Ph.D.

PROJECT OFFICER (NIEHS): E. R. Soares, Ph.D., Senior Staff Fellow

DATE CONTRACT INITIATED: June 26, 1975

CURRENT ANNUAL LEVEL: $76,500

PROJECT DESCRIPTION

OBJECTIVES: The scope of this contract is to develop an understanding of the mutagenic effects in mice of some known mutagens, as measured by mean and variance changes in polygenic characters.

METHODS EMPLOYED: The contractor proposes to investigate the cumulative effects of multiple mutagen-induced polygenic mutations on a series of traits on F_1 and F_2 generation progenies descended from mutagenized males of an inbred strain of mice. F_1 and F_2 progeny will be examined for a series of traits including: age of eruption of incisors, mean and variance of body weight at weaning, sex ratio at weaning, serum cholesterol level at weaning, and hematocrit at weaning. The contractor proposes to study at least two different mutagens in two strains of mice.

MAJOR FINDINGS AND PROPOSED COURSE: The completion of the statistical analyses of the initial year's work indicated that the mutagenic effects of TEM as measured by induced polygenic mutations were most apparent in progeny of treated sperm and spermatogonia. In order to confirm these findings and as planned in the original contract, this experiment is being repeated. Also, the first year's work showed some of the polygenic characters to be less applicable than expected. In this regard, some of the characters proposed initially have been replaced by other characters (e.g., cholesterol level at 6 weeks was replaced by a behavioral trait). The estimated length of this contract is 4 years.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the human environment there are many compounds known to be mutagenic. At present, no reliable assay exists for determining the rate of induction of point mutations in humans or, more generally, in mammals. Development of a polygenic test system could provide a means for detecting damage of this kind. Furthermore, once developed and understood, such a system would be economically feasible and could be coordinated with other mammalian mutagenesis test systems.

THE JACKSON LABORATORY - BAR HARBOR, MAINE (NIH-NO1-ES-4-2159)

TITLE: Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D., James Womack, Ph.D.

PROJECT OFFICER (NIEHS): Eugene R. Soares, Ph.D.

DATE CONTRACT INITIATED: February 19, 1974

CURRENT ANNUAL LEVEL: $42,000

PROJECT DESCRIPTION

OBJECTIVES: The sensitivity of a point mutation assay system is directly related to the number of loci that can be tested in each individual. In the present system in our laboratory, nine loci are sampled. Other inbred strains of mice possess enzyme variants which can possibly be incorporated into one of our present strains. This contract seeks to fully identify these additional variants (at least 13) and then be repeated backcrossing to incorporate them into the C57BL/6J genome.

METHODS EMPLOYED: Starch gel electrophoresis is used to determine whether a strain carries an allele that is not found in either the DBA/2J or C57BL/6J strains. When such a variant is found, the strain is backcrossed repeatedly to C57BL/6J for 8-10 generations to develop a new strain which will be homozygous with C57BL/6J. Electrophoresis tests will be performed on progeny in each generation to insure that the allelic differences already present in the C57BL/6J line are maintained.

MAJOR FINDINGS AND PROPOSED COURSE: Matings have been established to combine on a common background five alleles not previously found in this particular strain (C57BL/6J). Once homozygous for these alleles, these mice will be used as the strain onto which all other alleles will be placed. Backcrossing is continuing for all other loci in preparation for crosses onto the above strain.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: While many compounds in the environment have been shown to cause point mutations in lower organisms, fewer compounds have been tested in mammals due to the lack of a feasible test system. Also, a program to monitor the human population for the detection of electrophoretic mobility mutations is in the developmental stages. However, no comparable study has been conducted with any laboratory mammal; and hence, there is no base-line data for known mutagens for this type of mutation event. In an attempt to elucidate these areas, we are presently using two mouse strains that have nine different isozymes which can be detected electrophoretically. Since maximal sensitivity depends on polymorphic loci, it is desirable to increase the number of enzyme differences between the two strains. There are at least 13 other allelic differences which are present in various other strains or stocks of mice. If these were incorporated into one of our strains, this would increase greatly the sensitivity of the system

with a minimal increase in efforts. The strain will be made available for mutagenesis testing of environmental pollutants, carcinogens, and drugs to the scientists in the Laboratory of Environmental Mutagenesis.

THE JACKSON LABORATORY - BAR HARBOR, MAINE (NIH-NO1-ES-4-2156)

TITLE: Development of a System for Detecting Lethal Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): W. Sheridan, Ph.D.

DATE CONTRACT INITIATED: January 15, 1974

CURRENT ANNUAL LEVEL: $68,000

PROJECT DESCRIPTION

OBJECTIVES: At present no efficient test system exists for detection of point mutations in mammals. The aim of the contract is to develop strains of mice, carrying marked inversions, which may be used in studies of induction of point mutations. Theoretically these tests will be comparable to the system presently used in Drosophila. The prime goal is to induce inversions in as many chromosomes of the mouse as possible and combine them in a number of strains to be used as tester stocks.

METHODS EMPLOYED: The contractor induces chromosome breaks and inversions by means of radiation and/or chemical agents. He mates the treated animals and in the F_1 does cytological analysis of one testis of each individual male. If a significant increase in anaphase bridges appears, this is taken as an indication that the animal may carry an inversion; and he is mated to get offspring for further study. Following cytological and linkage studies to characterize the aberration, decision is made as to the utility of the inversion and appropriate marker genes are introduced into the stock.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor has induced and recovered 34 inversions. Of these, 19 have been thoroughly characterized for linkage and chromosomal morphology. One of the inversions has been successfully utilized in tests to detect induced recessive lethal mutations. The estimated length of the contract is five years. Efforts will continue to induce and characterize further inversions. It is proposed to combine inversions with Robertsonian Translocations to increase the effectiveness of the system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recessive lethal mutations are known to exist at high frequencies in human populations. Many environmental compounds are known to be mutagenic and capable of causing both chromosome aberrations and point mutations. With an efficient animal model to study the induction of such mutations, more accurate estimates can be made of the risks such compounds imply to the human population.

KNOX COLLEGE - GALESBURG, ILLINOIS (NIH-NO1-ES-5-2126)

TITLE: The Effects of Dietary Deficiency on the Sensitivity to Mutagenic Treatment

CONTRACTOR'S PROJECT DIRECTOR: Billy W. Geer, Ph.D.

PROJECT OFFICER (NIEHS): W. Sheridan, Ph.D.

DATE CONTRACT INITIATED: May 1, 1975

CURRENT ANNUAL LEVEL: $43,000

PROJECT DESCRIPTION

OBJECTIVES: In investigations using Drosophila melanogaster cultured on synthetic media deficient in vitamins and minerals, the frequency of chromosomal effects which were induced by irradiation was increased compared with treated cultures raised on non-deficient media. It is our purpose to investigate whether or not dietary deficiency might change the overall physiological state of the organism making it more susceptible to the actions of mutagenic agents.

METHODS EMPLOYED: Wild-type Drosophila are raised on a complete, defined, synthetic, germ-free medium. Subcultures have been placed on seven different media from which specific vitamins and minerals have been singly eliminated or depleted. Flies on deficient media and flies on whole medium have been exposed to similar doses of a known potent chemical mutagen. The frequencies of recessive lethal mutations and dominant lethal mutations are determined. Comparisons between the treated groups and with concomitant control groups are made in order to study any differences in the occurrence of induced genetic damage related to the diet.

MAJOR FINDINGS AND PROPOSED COURSE: Deficiencies for choline or cholesterol in the diet lead to greater sensitivity to mutagenic damage. Deficiencies for nicotinic acid or pyridoxine in the diet of males lead to reduced sensitivity, while no significant differences could be found when riboflavin or pantothenic acid deficiencies were studied. The contract expires May 1977.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A greater part of the human population on a world-wide basis is known to be subjected to nutritional insufficiency at the present time. At the same time, it is known that there are present in the human environment compounds which are mutagenic. Knowledge of the existence of effects of dietary deficiency on sensitivity to mutation induction will be a necessary consideration in attempts to extrapolate from information derived from laboratory animals in estimating risks to the human population.

MILES LABORATORY - Elkhart, Indiana
(NIH-NO1-3-2113)

TITLE: Genetic Tests to Characterize Specific Locus Mutants in Neurospora crassa

CONTRACTOR'S PROJECT DIRECTOR: J. L. Colbourn, Ph.D.

PROJECT OFFICER (NIEHS): Tong-man Ong, Ph.D., Geneticist, Microbial Genetics Section LEM

DATE CONTRACT INITIATED: April 9, 1973

CURRENT ANNUAL LEVEL: $70,000

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this contract is the genetic characterization of presumptive adenine-3 (ad-3) mutants of Neurospora crassa derived from forward-mutation experiments at the Institute and at Illinois State University (under contract with the Institute) with various environmental chemicals as well as various chemical carcinogens.

METHODS EMPLOYED: Stock cultures of presumptive ad-3 mutants are characterized by a series of heterokaryon tests with various standard tester sets which determine the genotypes of the mutants, the complementation pattern of ad-3B mutants showing allelic complementation, and also distinguish point mutations from chromosome deletions. The contract also includes making mutants homokaryotic and preparing silica gel stock cultures.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year more than 2500 mutants were made homokaryotic for adenine requirement, and silica gel stock cultures were prepared. Mutants which have been successfully characterized include those induced by 4NQO in haploid wild-type and in six different haploid UV-sensitive strains and those induced by various chemical carcinogens, nitrofurans, and various two-component heterokaryons. Mutants which will be analyzed in the future include (1) carcinogen-induced mutants in excision-repair deficient strains, (2) carcinogen-induced mutants in standard wild-type strains, and (3) carcinogen-induced mutants in the two-component heterokaryon homozygous for excision-repair deficient strains, as well as in heterokaryon 12.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to various programs in this Institute where it is essential to characterize the mutants induced at specific loci in this organism with regard to the type of lesion (e.g., point mutation or chromosomal deletion as well as the nature of the damage to the DNA at the molecular level). This is essential in our program to determine the correlation between carcinogenic and mutagenic activity as well as to determine whether chemical carcinogens

characteristically produce particular type of lesion at the molecular level. In addition, the characterization of the specific locus mutants produced by environmental chemicals enables us to better determine their relative hazard for man.

NEW YORK MEDICAL COLLEGE - VALHALLA, NY (NIH-NIEHS 75-29)

TITLE: Tier One Microsomal Assay for Mutagenesis

CONTRACTOR'S PROJECT DIRECTOR: Herbert S. Rosenkranz, Ph.D.

PROJECT OFFICER (NIEHS): Larry Claxton, Biologist

DATE CONTRACT INITIATED: March 11, 1975

CURRENT ANNUAL LEVEL: $113,481

PROJECT DESCRIPTION

OBJECTIVES: The purposes of this project are (1) the testing of environmental compounds for mutagenicity in bacteria in the presence of mammalian microsomal enzyme preparations, (2) evaluation of the use of a computerized testing format and data analysis and (3) evaluation of the feasibility of mass screening using the microsomal bacterial assay.

METHODS EMPLOYED: Highly trained technicians are following computerized protocols in the testing of environmental chemicals in a blind study. The data analysis will be done via a computerized system and the feasibility of this system will be evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: The contract is in progress. Since this is a blind study, however, the data analysis has not been done at present. Efforts will be made to conclude this pilot project and to evaluate the data generated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The applicability of computerized testing systems in mutation testing will be determined. Large scale screening in its initial phases will also be evaluated. Environmental chemicals of health and economic importance will be tested.

INFORMATION CENTER COMPLEX/INFORMATION DIVISION
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE 37830
(Interagency Agreement Between NIEHS and ORNL)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTE: National Cancer Institute; Energy Research and Development Administration

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $190,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information.

MAJOR FINDINGS AND PROPOSED COURSE: EMIC's data file at the end of March 1977 contained 19,000 bibliographic entries. Of these, approximately 18,000 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number(s). The Center processes requests for information at a rate of 2-3 per day. These requests come primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received. The EMIC data bank can now be screened by ToxLine. EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objectives as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information from the scientific, education, and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with the timely state-of-art reports on subjects of current scientific interest.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators, and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (1550). EMIC has helped several national commissions in collecting and extracting the

data from the literature. For the Institute, and especially the Laboratory of Environmental Mutagenesis, EMIC saves more than one man-year of time for the personnel in the Laboratory by supplying their information needs.

PUBLICATIONS

Wassom, J. S., Shelby, M. D., Von Halle, E. S., Owens, E. T., Brooksbank, R.E., Jr., Miller, I. R., Beauchamp, R. O., Jr., Barnard, W. J., and Adams, J. O. Chemical Mutagenesis in Laboratory Mammals - A Bibliography on the Effects of Chemicals on Germ Cells. Oak Ridge National Laboratory Publication No. ORNL/EMIC-6. Oak Ridge National Laboratory, Oak Ridge, TN, 1976, 105 pp.

Shelby, M.D. and the Environmental Mutagen Information Center Staff. Chemical Mutagenesis in Plants and Mutagenicity of Plant Related Compounds. Oak Ridge National Laboratory Publication No. ORNL/EMIC-7, Oak Ridge National Laboratory, Oak Ridge, TN, 1976, 327 pp.

Wassom, J. S., Shelby, M. D., Von Halle, E. S., Beauchamp, R. O., Jr., Owens, E. T., Miller, I. R., Barnard, W. J., and Adams, J. O. Chemical Mutagenesis: A Survey of the 1974/1975 Literature. Oak Ridge National Laboratory Publication No. ORNL/EMIC-8. Oak Ridge National Laboratory, Oak Ridge, TN, 1976, 311 pp.

Malling, H. V. and Wassom, J. S.: Action of Mutagenic Agents. In Wilson, J. G. and Fraser, F. Clarke (eds.): Handbook of Teratology. New York, Plenum Press, 1977, pp. 99-152.

Wassom, J. S., Shelby, M. D., and Von Halle, E. S.: Mutagenesis and Teratogenesis Information Programs. In, Proceedings of the Symposium on the "Handling of Toxicology Information," Bethesda, MD. (in press)

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON
(NIH-NIEHS-74-2151)

TITLE: Detection of Point Mutations in Somatic Cells

CONTRACTOR'S PROJECT DIRECTOR: Peter E. Nute, Ph.D.

PROJECT OFFICERS (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED; January 1, 1974

CURRENT ANNUAL LEVEL: $167,000

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many different types of hemoglobin mutations exist in the human population. Some of these are point mutations in the α- and β-chain. Since such mutations occur in the germinal tissue, it is reasonable to assume that they also occur in the stem cells for the red blood cells (RBC). This type of mutation should result in an RBC which would contain an aberrant hemoglobin. By using a monospecific antibody to a number of aberrant hemoglobin types, such cells should be detected in samples from normal individuals after reaction of the antibodies with various fluorescent dyes. Antibodies are being produced against HbS, HbC, HbOArab, HbE, HbQ India, Hb Ottawa, Hb Hasharon, Hb Cranston, Hb Wayne, and Hb Constant Spring.

MAJOR FINDINGS AND PROPOSED COURSE: Fluorescent monospecific antibodies to Hb-S have been produced. Reconstruction experiments have shown that by use of anti Hb-S one Hb-S cell can be detected among 10^6 normal cells. In normal human the frequency of Hb-S containing cells range at approximately 10^{-7}, which is low enough to allow for a positive detection of mutagenic effects. Hb-S and Hb-C are mutants with different amino acids incorporated at the same place (#6) in the β-hemoglobin. The monospecific antibodies to Hb-S do not cross-react with the Hb-C containing cells and vice versa.

The Hb-S and Hb-C antibodies will be used to investigate somatic cell mutation rates with respect to age and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect mutations in the genome of readily accessible somatic cells

would allow estimation of the mutation rate in the human population. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PUBLICATIONS

Papayonnopoulou, P. H., Lim, G., McGuire, T. C., Ahein, V., Nute, P. E., and Stamatoyannopoulos, G.: Use of specific fluorescence antibodies for the identification of hemoglobin C in erythrocytes. American Journal of Hematology. (in press)

Papayonnopoulou, P. H., Nute, P. E., Stamatoyannopoulos, G., and McGuire, T. C.: Hemoglobin Ontogenesis: Test of the gene excision hypothesis. Science (in press)

LABORATORY OF ENVIRONMENTAL TOXICOLOGY

LABORATORY OF ENVIRONMENTAL TOXICOLOGY
Summary Statement

The scientific efforts of the Laboratory of Environmental Toxicology (LET) are directed toward the prevention of environmentally-related diseases through understanding mechanisms of toxicity. Special emphasis is placed upon environmental factors which may adversely affect reproduction and development. Toxicologic phenomena are studied from the descriptive to the molecular level in search of a better understanding of mechanisms. This increased understanding contributes to the development of better and more effective test procedures to predict toxicity in humans. Available scientific expertise is broad and includes biochemistry, reproductive physiology, teratology, pharmacology, cell biology, pathology, and behavior. Test systems are likewise diverse and range from molecular biochemistry and electron microscopy, through in vitro cell, organ, and embryo cultures, to whole animals. Whether their study be molecular and/or biochemical mechanisms of toxic action, pharmacology and pharmacokinetics in developing systems, the identification of sensitive biochemical indicators which predict specific organ damage, or some other area of toxicology, scientists in LET are in a unique position to develop and utilize basic scientific information for resolving critical environmental health problems.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving both national and international programs and adjunct appointments. Recognizing training as one of its primary responsibilities, the Laboratory supports graduate students at nearby universities, offers training opportunities for foreign postdoctoral scientists and American scientists on sabbatical leave. In short, the Laboratory of Environmental Toxicology integrates research and training into a program that is both worldwide in scope and one of the strongest multidisciplinary efforts in toxicology in this country.

EXPERIMENTAL TERATOGENESIS

The research of this group seeks a better understanding of teratogenic compounds, particularly their molecular mechanisms of action. Scientists are currently testing the hypothesis that certain drugs and environmental chemicals interfere with embryogenesis by irreversibly combining with critical molecules in the fetus. As a first step to investigate this possibility, known acylating agents of simple chemical structure, including anhydrides and imides, are being used as test compounds. To determine the teratogenicity and embryotoxicity of these agents, each is administered to pregnant CD-1 mice. One day prior to full term, fetuses are removed and examined for gross, visceral or skeletal abnormalities. All of the acylating agents thus far tested have produced malformations in the offspring of treated dams.

In order to correlate teratogenic potential with chemical reactivity, the interaction of acylating agents with fetal and maternal macromolecules *in vivo* and proteins and model nucleophiles *in vitro* is being studied. Following the administration of ^{14}C phthalic anhydride, covalently bound radioactive material has been found in the tissues of both the mother and the conceptus.

TERATOLOGY

This group is concerned with the environmental agents that cause birth defects in mammals. A large part of this group's effort is devoted to the development of an Environmental Teratology Information Center (ETIC), providing the biomedical community access to all publications that contain data pertinent to the teratogenic potential of environmental agents or conditions to which man is exposed. This program will help in defining priorities for future investigations and in preventing unplanned duplication of research. The first block of information is now available on-line as a part of the National Library of Medicine's ToxLine.

Modern man is exposed continuously to a multitude of physical as well as chemical and infectious stresses. Most of these agents have not been tested for their teratogenic potential. In collaboration with scientists of the Laboratory of Environmental Biophysics, scientists have exposed mice to jet engine and "sonalert" noise. More than 3500 women of child-bearing age in the United States alone are currently being exposed to this type of noise at up to 130 dB. These types of noise, either singly or in combination, have not been found to cause teratogenicity in mice.

Simultaneous exposure to two or more chemicals represent a more realistic environmental exposure; and the toxicity of combined chemicals might be greater than their additive toxicities. In this regard, carbaryl, a widely used insecticide, was administered to the mouse alone and in combination with piperonyl butoxide or phenobarbital. No positive teratogenicity was detected. In other studies, the administration of carbon monoxide with ethanol or with sulfur dioxide did not result in a teratogenic response in either rabbits or mice. In another project, scientists in this group and contractors at the Research Triangle Institute have detected teratogenicity following oral administration of polychlorinated biphenyls to mice. As part of the US-USSR Cooperative Program, a scientist in this group studied the teratogenic potential of the insecticide Dipterex in the rat, mouse, and hamster. Dipterex was teratogenic in all three species when given three times daily by gavage.

MOLECULAR EMBRYOLOGY

This workgroup is concerned with defining and analyzing certain molecular aspects of the differentiation process. In particular, research concentrates on analyzing patterns of gene expression at the transcriptional and translational level in the model embryoid body/teratoma system (OTT 6050) in the mouse. In this system, the embryoid bodies, which consist of an outer ring of endoderm and an inner mass of embryocarcinoma cells, grow as an ascites in mice. Frequently, embryoid bodies "implant" on the liver, spleen, or mesentary and this stimulates the production of teratomas, predominately neuroepithelial.

Using oligo-dT cellulose chromatography, the approach has been to isolate total poly(A)-containing RNA from embryoid bodies, teratomas, and the F9 embryocarcinoma cell line grown in vitro. These RNA preparations are then characterized with respect to poly(A) content, molecular size, and length of the poly(A) tract. Complementary DNA probes are prepared to these RNA preparations using reverse transcriptase and nucleic acid hybridization techniques are used in analysis. The sequence and abundance of classes of these preparations are being defined. Results indicate that the RNA sequence populations in both embryoid bodies and teratomas can be divided into three classes, low, middle, and high complexity, with the tumors expressing 2-4 times more information in each RNA sequence class.

Another area of research involves the development of in vitro translation systems for use in isolating and purifying specific messenger RNAs. Preliminary data on in vivo patterns of protein synthesis suggest some of the major proteins synthesized in the embryoid bodies and teratomas. With this information, the potential messenger RNAs in the system can be identified and the in vitro translation system utilized to identify and purify these messenger RNAs. With particular messenger RNAs specific for the embryoid bodies or teratomas, specific complementary DNA probes can now be made for the analysis of gene expression in an in vitro chromatin/RNA polymerase system. Efforts are being made to define those molecules in chromatin involved in specific gene expression during the differentiation process.

TRANSPLACENTAL TOXICOLOGY

This group has demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring of mice. Such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β-estradiol. Differential fetal protein binding between DES and estradiol may help explain these results.

Scanning electron microscopy (SEM) of the genital tract of in utero exposed females has revealed surface ultrastructural abnormalities which may precede neoplastic changes. SEM may provide a tool for early detection of such changes. These studies are being correlated with observations on the role of steroid receptors in reproductive tissue alterations. In studies with male offspring from DES treated mice, a high incidence of testicular and sperm abnormalities was detected. The fate of Mullerian duct remnants in DES treated males is being determined and the role of these tissues in prostatic lesions evaluated. The distribution, metabolism, and structure-activity relationships of DES in perinatal systems have been determined. The role of toxication-detoxication processes is being evaluated in specific reproductive tract lesions. These studies emphasize the biological significance of potentially activated metabolites. These studies should aid in extrapolating these results to other classes of estrogenic environmental chemicals.

DEVELOPMENTAL PATHOLOGY

This program seeks to determine the postnatal toxic effects on offspring of rats exposed to chemicals throughout gestation. Offspring are evaluated at various stages of development from newborn to geriatric. In addition to gross observations, the disposition of the chemical as well as effects on organ system function, immunological competence, learning and behavior are determined. Gross, histological, and subcellular pathology in offspring of treated and control females is analyzed. The offspring's adaptation ability and endurance capacity are also determined. Current studies demonstrate that methyl-n-butyl ketone (MBK) and metabolites are present in both the fetal and maternal compartments in term pregnant rats. MBK exposed females exhibited a 12 to 24 hour delay in parturition. Offspring of exposed animals, although similar at birth, show a progressive delay in development at all stages tested during the first six months. Biochemical changes and the mechanisms responsible need to be identified. Acute toxicity studies with methylglyoxal have shown that pregnancy protects against the toxic effects of the chemical. A possible explanation has been postulated. Other chemicals such as enflurane and ethchlorvynol have also been found to cause biochemical and behavioral changes during early postnatal development following gestational exposures. The characterization of potential organ pathology in offspring exposed in utero and the delineation of the total spectrum of postnatal toxicity as well as the mechanisms responsible for these effects are challenging areas of study in environmental health science.

GERM CELL DEVELOPMENT

Group scientists are investigating the effects of environmental chemicals on germ cell toxicity. New approaches to reproductive toxicity testing are being explored and validated while other investigations are directed towards the elucidation of mechanisms of toxicity. Rat testes are used to study (1) DNA repair synthesis and DNA breaks as an indicator for chemical mutagens; (2) toxication-detoxication reactions of polycylic hydrocarbons in gonadal tissue; and (3) toxic effects on reproductive function.

Alkaline elution analysis is a sensitive method for detecting DNA single strand breaks and/or cross links in mammalian germ cells. Kinetics of alkaline elution of DNA single strand breaks, stabilization of DNA and/or increased DNA repair synthesis in germ cells after chemical exposure were found to be reliable indicators of toxicity. Recent investigations of testicular microsomal and soluble enzymes demonstrated that both aryl hydrocarbon activating and deactivating enzymes were present. Developmental patterns of these enzymes in rat testes indicated that glutathione S-transferase activity in prepubertal rats was 3-4 times greater than that of the liver enzyme. In contrast, epoxide hydrase activities of both liver and testes were very low in prepubertal rats but dramatic increases were evident at the onset of puberty. In vitro perfusion of the rat testis with tritium labeled benzo(a)pyrene 4,5-oxide indicated that the active benzo(a)pyrene metabolite was quickly conjugated or converted to a dihydrodiol derivative. The rates of detoxication via these two pathways suggested first order kinetics; the reactions were concentration dependent. Furthermore, in vitro perfusion of testis provides a unique model system for the integration of

molecular, biochemical and physiological data with pharmacokinetic parameters. This approach for the study of environmentally-induced alterations of male reproductive organs should serve as an important model for extrapolation to human problems.

DEVELOPMENTAL TOXICOLOGY

This group is concerned with perinatal aspects of enzymology, endocrinology, and pharmacology. A multidisciplinary effort is utilized in attempts to identify sensitive biochemical indicators of developmental toxicity having clinical value and to understand the mechanisms responsible for alterations in normal enzyme development. Emphasis is placed on the hormonal regulation of enzyme development in control and treated animals and the role of altered hormone action in developing systems. Biochemical and morphological changes are correlated with the uptake, binding, metabolism, accumulation, and clearance of foreign compounds and hormones in whole animals and culture systems. Current interests center on liver, intestine, reproductive tract, and the pituitary-hypothalamic axis.

The specific fetal and newborn tissue distribution of several chlorinated and brominated biphenyls has been studied. Pregnant rats were administered labeled PCBs during various gestation periods and levels of radioactivity measured in a broad spectrum of maternal and fetal tissues and fluids. One surprising finding was that glucuronides of hydroxylated PCBs accumulate dramatically in fetal intestine but these levels were rapidly depleted following birth. 2,4,5-2',4',5'-hexachlorobiphenyl (6-CB), which does not accumulate in the fetal intestine, is secreted in the milk although significant newborn tissue levels of this isomer also result from placental transfer. Significant levels of 6-CB were detected in brown fat immediately after birth. The brown fat is rapidly depleted causing a redistribution of 6-CB to other tissues. 2,4,5-2',4',5'-hexabromobiphenyl (6-BB) crosses the placenta slowly but is secreted in high concentrations in milk. Further experiments are investigating the relative importance of placental transfer and milk secretion of 6-CB and 6-BB following treatment of female rats two weeks prior to mating.

The effect and mode of action of hormones and xenobiotics on the regulation of enzyme patterns during perinatal development is also being investigated. The postnatal development of rat liver histidase 5α-reductase, 16α-hydroxylase and several other organelle specific enzymes following perinatal exposures to diethylstilbestrol (DES), zeranol, the PCBs, testosterone and estradiol is being defined. Rat liver histidase and 5α-reductase undergo a complex postnatal developmental course resulting in enzyme levels which are considerably higher in the adult female than the male. Recent experiments have demonstrated that prenatal exposure to DES does not alter hepatic histidase activities in immature male and female offspring and adult male offspring. However, histidase activities of adult female rats were decreased by 30% approaching activities of adult males. Thus, DES exerts an apparent programming effect on hepatic histidase following prenatal exposures. In contrast, the perinatal development of 5α-reductase is unaffected by prenatal exposure to DES although a programmed response is evident when DES is administered during the first week after birth.

Another area of research focuses on hormonal and environmental factors affecting intestinal function and energy-producing processes during perinatal development to identify biochemical components indicative of normal and/or altered intestinal function. One study has shown that intestinal pyruvate dehydrogenase (PDH) is converted to its active form immediately after birth and that this conversion is correlated with concomitant changes in the mitochondrial redox state. Activation of PDH during the early postnatal period allows for increased utilization of pyruvate by the TCA cycle resulting in increased ATP production necessary for active transport to occur in the developing intestine. PDH activity and the activation process are highly sensitive to oral arsenic exposures. β-glucuronidase (βG) activities are extremely high in the perinatal period and since fetal and newborn tissues often exhibit enzyme activities similar to those of tumor tissues, newborn intestinal isozyme profiles were compared to those of normal adult rats and rats exposed to dimethylhydrazine, a specific colon carcinogen. Preliminary results indicate that βG may provide an early enzyme indicator for intestinal carcinogenesis. An intestinal primary culture system formed by the isolation of tip and crypt cells is being developed and evaluated as a possible in vitro model for cellular differentiation, intestinal toxicity, and intestinal toxication-detoxication of environmental chemicals.

BIOCHEMICAL TOXICOLOGY

Scientists in this workgroup study basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in mammalian tissues. The goal is to apply this information to designing rapid and effective test procedures for predicting toxicity in man.

Factors affecting hepatic heme synthesis and its use during mammalian development are being studied. Differences in regulatory and biochemical properties of several heme biosynthetic pathway enzymes in adult and fetal liver including those of α-aminolevulinic acid (ALA) synthetase, which is considered to be rate-limiting in this process, are being clarified. These studies also showed heme plays an important role in regulating synthesis of various mitochondrial proteins in fetal mammalian liver. This action is probably mediated through a direct stimulatory effect of heme on the synthesis of apocytochrome oxidase and, perhaps, other hemoproteins which comprise integral portions of the mitochondrial structure. Because of the important regulatory role played by heme during fetal hepatic development, it is likely that late gestation may be a period of enhanced susceptibility to effects of environmental agents which selectively interfere with heme biosynthesis or its utilization. Studies are currently in progress to assess effects of prenatal exposure to hematotoxic agents on the postnatal development of hepatic hemoprotein function.

Other scientists are attempting to define the early ultrastructural and biochemical effects of heavy metals and other environmental agents on mammalian tissues to derive early warning tests for predicting sub-toxic human responses to such agents. Their studies include determining the correlation between subcellular morphologic observations and biochemical alterations in tissues of animals chronically exposed to low levels of toxic agents for various periods. In current studies, researchers are examining the toxicity of

arsenic, cadmium, lead, methyl and inorganic mercury, dioxin (TCDD), and vinyl chloride. Results of their experiments indicate that specific morphological and biochemical response profiles can be established which differentiate various metals and other agents in our multi-element environment. Studies are currently in progress to use these profiles to develop test procedures that will permit identification of preclinical exposure to specific environmental substances in human populations.

BEHAVIORAL TOXICOLOGY

NIEHS is developing a behavioral toxicology program in an attempt to define methods for determining what environmental stresses produce behavioral alterations in mammals and lower species. As part of this effort, scientists are developing model test systems which simulate and predict behavioral toxicity in humans and are considering neurochemical mechanisms of behavioral toxicity. Some research in this area involves the neurotoxicological effects of hexabrominated biphenyl compounds in male and female rats and mice receiving various doses for periods up to six months. In another series of experiments, an attempt is being made to determine the various factors in the etiology of pica (the consumption of seemingly non-nutritive substances) and to validate the utility of pica as a behavioral assay of toxicosis. A third study investigates the consequences of inhibiting monoamine oxidase A and B on different aspects of behavior. This research has consequences with respect to several pesticides which are known to inhibit monoamine oxidase.

In the coming year, the Laboratory of Environmental Toxicology will continue to expand its research activities in each of the areas described. It will develop a more systematic routine testing capability, as well as validate and improve test methods. Additionally, the Laboratory will attempt to extend laboratory animal observations to the human population. In this effort, it will encourage an increasingly strong interaction between laboratory scientists, clinicians, and epidemiologists.

COLLABORATIVE RESEARCH

NIH CONTRACTS

Laboratory scientists are Principal Investigators in a wide variety of research contract efforts. These collaborative efforts are logical extensions of the Laboratory's intramural programs concerned with reproductive, developmental, and target organ toxicities.

Dr. Staples has contracted with DOW Chemical Company and the Research Triangle Institute to determine the teratogenic potential of environmental agents in animals. Test agents are applied singly or in combination to more than one species in an effort to discover teratogenic effects as well as possible synergistic actions and/or susceptible populations. Experiments are designed to increase the resolving power of tests which detect potential teratogenic hazards to humans.

Drs. Lucier and McLachlan are collaborating with the Endocrine Labs of Madison to search for the transplacental toxicity of selected environmental chemicals. This contract emphasizes reproductive tract function and transplacental carcinogenesis and fulfills a critical need to identify chemicals or classes of chemicals that might adversely affect reproductive tract function following gestational exposures. These chemicals can then be studied in detail in the intramural program, focusing on the mechanisms of toxic action. Chemicals currently under study include zeranol, several PCB and PBB isomers, DDE, and a series of mycotoxins.

Dr. Lee has been assessing the effects of environmental agents on male reproductive tract function in a contractual research effort with Hazleton Laboratories. These efforts seek to define the reproductive toxicity of metals such as boron, cadmium, aluminum, and lead. Reproductive tract function is monitored by serial mating, hormone levels, blood chemistry, and histopathology.

Dr. Fowler has recently initiated a contract with the University of Washington to investigate the potential carcinogenic actions of petroleum hydrocarbons using selected estuarine organisms as the model. This research is exploring the relationship between tumor induction and metabolism of the chemical carcinogen, benzo(a)pyrene. The exposure system has been developed, the microsomal enzyme assay system optimized, and the tumor biopsy procedure refined.

Dr. Woods is extending the Laboratory's Target Organ Toxicity efforts through a research contract with Midwest Research Institute. Animals exposed chronically to vinyl chloride (VC) and vinylidene chloride (VD) are evaluated and general toxicity and carcinogenic potential defined. Data derived from these studies are being utilized in the design of models for risk assessment in humans.

Two new contracts will be awarded during FY 77. Dr. Lucier seeks to identify clinically-useful biochemical indicators for developmental toxicity. Dr. Woods will investigate the effects of long-term, low-level exposures to various metals on heme biosynthesis and metabolism in rhesus monkeys seeking to design predictive tests for assessing sub-toxic responses to heavy metals in human populations.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international level. Laboratory scientists have acknowledged the resources and expertise concentrated in nearby universities. Moreover, we have not hesitated to establish the collaborative efforts necessary to develop quality intramural programs with scientists throughout the world, including government, academia, and private industry.

Dr. Woods and the NIEHS Biometry Branch are collaborating with the Department of Epidemiology, UNC School of Public Health on the utilization of animal toxicity data in the design of models for carcinogen risk assessment in human populations exposed to vinyl chloride.

Dr. Fowler and Dr. Krigman of the UNC Pathology Department are studying the subcellular distribution of lead in experimental animals in relation to mechanism of toxicity. Dr. Fowler and NOAA scientists are also investigating the physiological effects of arsenic, cadmium, and copper on marine shellfish. This effort is directed at elucidating the mechanisms by which commercially-important marine shellfish concentrate heavy metals. An unusual cadmium-binding protein has been isolated from shellfish, an increased incidence of copper-containing lysosomes noted in copper-treated shellfish tissues, and the toxic actions of cadmium and copper on gill respiration are being examined. In addition, Drs. Fowler and Nordberg (Karolinska Institute) are investigating the toxic manifestations of cadmium exposures at a subcellular level.

Dr. Lucier is studying the role of hormone receptors in xenobiotic induced alterations of imprinting with Dr. Shihadeh Nayfeh of the UNC Department of Biochemistry. This project should yield information important to understanding the mechanism of action of important forms of developmental toxicity. In a related project, Drs. Lucier, Lamartiniere, and Fiegelson (Roosevelt Hospital, New York) are attempting to utilize hepatic histidase as a marker indicative of perinatal imprinting of hepatic metabolism.

Dr. McLachlan has several collaborative projects involved with a detailed exploration of the transplacental toxicity of DES and other hormonally-active chemicals. He and the UNC Cancer Research Center are utilizing SEM as a tool to detect early preneoplastic lesions in animals exposed to DES during gestation. Research projects with Dr. Gerald Cunha of the University of Colorado Medical Center, and Dr. Manfred Metzler of the University of Wurzburg, West Germany, are attempting to identify pharmacological properties and cellular alterations important to unravelling the mechanism of DES-induced transplacental carcinogenesis.

Dr. Lee is studying the toxic actions of trace metals and their effects on plasma gonadotropins and androgens. In an international collaboration, Dr. Lee and scientists from the Institute of Toxicology and the University of Zurich are developing a new method for accurately identifying mutagens and carcinogens through an in vivo animal model.

Dr. Dixon has developed a productive collaboration with Dr. Sergio Fabro of the Columbia Hospital for Women. Dr. Fabro has been active in the Experimental Teratology group and this effort is directed at determining mechanisms of teratogenesis.

Drs. Dixon, Staples, Lee, and McLachlan are also involved in the US-USSR collaborative research programs in environmental health concerning transplacental toxicity and chronic organ toxicity.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer approval and scientific relevance of current Laboratory programs is the frequency that LET scientists organize and participate in "state of the art" symposia and are asked to serve on

various committees attempting to provide meaningful direction in environmental health research. Descriptions of representative examples of Laboratory involvement in this regard are given below.

Dr. Dixon has a large number of committee and program assignments which augment and are relevant to the NIEHS mission such as the Pharmacology-Toxicology Research Committee of the NIGMS, Chairman of the Committee on Environmental Pharmacology of ASPET, Committee on Women in the Workplace organized by NIOSH, Project Director of areas 2 and 4 of the NIEHS Energy-Related Research Program, US-USSR Problem II Coordinator of the Joint Committee for Health Cooperation-Environmental Health involving chronic organ toxicity, Councilor of the Pan-American Medical Association-Section on Environmental Health Sciences, and the Toxicology Review Panel of WHO's Expanded Programme of Research Development and Research Training in Human Reproduction. In addition, Dr. Dixon has been co-organizer of several Target Organ Toxicity Symposia in cooperation with the Society of Toxicology. Proceedings of these symposia are published in Environmental Health Perspectives and include symposium on liver, kidney, lung, gonads, and development.

Dr. Lucier is the NIEHS representative to the NIH Digestive Diseases Coordinating Committee. Dr. Schiller has a similar appointment on the Nutrition Coordinating Committee. She is also chairman of the committee on In Vitro Research and Human Values of the Tissue Culture Association and recently organized a symposium on the Scientists' Responsibilities in the Acquisition and Utilization of Human Tissue for In Vitro Research. In addition, Dr. Schiller is a member of the Advisory Committee of the NIEHS Federal Women's Program.

Dr. Woods serves on DHEW's "Toxicology Information Subcommittee" which coordinates toxicology and related programs in DHEW. Moreover, he is a member of the Federal Working Group's research panel on Pest Management. Dr. Fowler has been extremely active in National and International Programs dealing with metal toxicity. He was a rapporteur at a recent conference held at the Karolinska Institute entitled "Air Pollution and Cancer: Risk Assessment Methodology and Epidemiology Evidence" and was a co-organizer of an International Conference on Arsenic Toxicity held in Fort Lauderdale, Florida. Dr. Fowler is serving on the ERDA "Health Effects Panel on Trace Contaminants from Coal Combustion Systems."

Dr. McLachlan is a member of the HEW committee on DES toxicity and the significance of his intramural research effort was recognized by selection as an expert witness at the recent DES hearings.

Dr. Staples' pioneering work involving communication of teratological information has led to interactions with ERDA, NLM, NBS, and NICHD. He is Secretary and Councilor-elect for the Teratology Society and was recently named as liaison scientist between the Teratology Society and the Society of Toxicology. Dr. Staples is the U.S. representative for Teratology in the US-USSR Collaboration on Environmental Health.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. In this regard, Dr. Staples has established the much-needed Environmental Teratology Information Center (ETIC) in collaboration with ERDA and Oak Ridge. Information on teratology is collected, categorized and made available to the biomedical community via TOXLINE. Dr. Staples is also on the Editorial Boards of Environmental Health Perspectives and the Journal of Environmental Health Sciences. Dr. Lucier is the editor of Environmental Health Perspectives. Dr. Dixon is on the Editorial Boards of Environmental Health Perspectives, Toxicology and Applied Pharmacology, Journal Of Pharmacology And Experimental Therapeutics, and the Journal Of Environmental Sciences And Health. In addition, LET scientists are frequently asked to review manuscripts for journals oriented toward Biochemistry, Pharmacology, Toxicology, and Teratology.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Environmental Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training programs, including adjunct appointments at nearby universities, supervision of graduate student research, development of graduate courses in environmental health, and participation in the Fogarty Visiting Program.

Several LET scientists have adjunct appointments with nearby universities. Dr. C. Mitchell is Adjunct Professor in the Pharmacology Department of UNC School of Medicine; Dr. Lucier is Adjunct Associate Professor in the Biochemistry Department of the UNC School of Medicine; Dr. Staples is Adjunct Associate Professor of Pharmacology in the Pharmacology Department of the UNC School of Medicine; Dr. Fowler is Adjunct Assistant Professor in the Pathology Department of the UNC School of Medicine; Dr. Schiller is Adjunct Assistant Professor of Biochemistry in the UNC School of Medicine and is also on the faculty of the W. Alton Jones Cell Science Center, Lake Placid, New York. Dr. D. Mitchell is a Visiting Scientist in the Zoology Department of Duke University.

Drs. Schiller and Lucier are involved in organizing a graduate course in Biochemical Toxicology to be taught by the UNC Biochemistry Department.

Laboratory scientists have also been active in the training of graduate students. Two students are working in Dr. McLachlan's laboratory and Drs. Schiller and Lucier are supervising the graduate research of Winifred Curley (UNC Biochemistry Department). Postdoctoral training is strongly supported by LET and our commitment to these programs involves the support of nine Visiting Fellows, two Visiting Associates, and one postdoctoral fellow. In addition, Dr. Kirk Kitchin is a guest worker from the Chemical Industry Institute for Toxicology (CIIT) currently working with Dr. Woods.

The interagency transfer of funds process has allowed Dr. Zeller (Northwestern University) and Dr. Sanyal (Columbia Hospital for Women), Dr. Lamb (UNC Cancer Center) and Dr. Davidian (UNC Biochemistry Department) to collaborate with NIEHS scientists in our intramural research effort.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70001-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Teratogenicity of Acylating Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Dixon	Chief	LET NIEHS
OTHER:	S. E. Fabro	Consultant	Columbia Hosp.
	N. A. Brown	Visiting Fellow	LET NIEHS
	J. Kao	Visiting Fellow	LET NIEHS

COOPERATING UNITS (if any)
Columbia Hospital, The George Washington University

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Experimental Teratogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

We hypothesize that acylation is a mechanism of chemical teratogenesis. If acylation is a mechanism of teratogenicity, then acylating agents such as snhydrides (and imides) should be teratogenic. The embryototoxicity and teratogenicity of a variety of anhydrides (acetic, propionic, succinic, maleic, and phthalic) and succinmide were evaluated following intraperitoneal administration of gestations days 8, 9, and 10 or 11, 12, and 13. All the compounds tested were both embryotoxic and teratogenic. The most common congenital abnormalities induced by treatment on days 8, 9, and 10 were rib and vertebral malformations. Treatment on gestational days 11, 12, and 13 produced predominantly cleft palate. In vivo studies indicated that phthalic anhydride was bound to macromolecules of maternal liver, lung, kidney, and spleen as well as the uterus, placenta and fetuses. These results suggest a correlation of these acylating reagents between their teratogenic potential and their chemicobiological interactions.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant CD-1 mice received selected anhydrides or imides by i.p. injection on either days 8, 9, and 10 or days 11, 12, and 13 of gestation. The chemicals were suspended in 0.5% methylcellulose and administered soon after preparation. Controls received only the vehicle. Mice were sacrificed by cervical dislocation on day 18 of gestation (copulatory plugs being Day 1). The young were delivered by Cesarean section, designations made between live and dead fetuses, and resorptions counted. All live young were weighed and examined for grossly observable malformations. The viscera of each were examined by a soft tissue visceral technique described by Staples and the sex noted. The heads were fixed in Bouins for examination by Wilson's free hand slicing. Some fetuses were then cleared and stained by the KOH-Alizarin Red S method for skeletal examination.

Covalent binding *in vivo* and *in vitro* was assessed using various C^{14} labeled acylating agents. Binding was determined by the method of Mitchell and Jollow which involves a series of TCA and methanol wastes to remove unbound radioactivity. Some samples were also washed with methanol in a Soxhlet extractor.

MAJOR FINDINGS AND PROPOSED COURSE: The embryotoxicity and teratogenicity of a variety of anhydrides (acetic, propionic, succinic, maleic, and phthalic) were evaluated following intraperitoneal administration on gestation days 8, 9, and 10 or 11, 12, and 13. Each anhydride tested was both embryotoxic and teratogenic. The most common congenital abnormalities induced by treatment on days 8, 9, and 10 were rib and vertebral malformations. Treatment on gestational days 11, 12, and 13 produced predominantly cleft palate. Succinimide was also demonstrated to be embryotoxic and teratogenic. Binding of the test chemicals to macromolecules was studied *in vivo* and *in vitro*. *In vitro* studies demonstrated covalent binding to various purified proteins. *In vivo* studies indicated that phthalic anhydride was bound to macromolecules of maternal liver, lung, kidney, and spleen as well as the uterus, placenta and fetuses. These results suggest a correlation between these acylating reagents, teratogenic potential and chemicobiological interactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We hypothesize that acylation is a mechanism of chemical teratogenesis; to test this idea, the teratogenicity of selected anhydrides and imides is being studied. If acylation is a mechanism of teratogenicity, then acylating agents such as anhydrides (and imides) should be teratogenic. Additionally, their teratogenicity should correlate with their spontaneous reactivity; covalent binding of anhydrides (and imides) to fetal macromolecules should be demonstrated. Our preliminary results suggest that this hypothesis is valid and could become an important factor in defining chemicals which have a teratogenic potential. These studies also contribute greatly to an understanding of molecular mechanisms which account for birth defects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70010-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Postimplantation Embryo Culture: A Model for the Study of Organogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. K. Sanyal Reproductive Physiologist LET NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Germ Cell Development Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↳

With the perspective of detection and study of mechanisms by which environmental agents induce developmental disorders, an in vitro culture system of embryos during organogenesis phase of development in the rat (pregnancy day 11) paralleling in vivo development has been established. In this culture system, the embryos grow 3-4 times their original size in 24 hours as assessed grossly, and by DNA and protein content. The major organs, brain, neural tube, sensory organs, circulatory system, gut, liver and masculature in the cultured embryos differentiate comparable to that in vivo.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat embryos are being cultured during the organogenesis stage of development. Conceptuses of pregnancy days 10 and 11 with embryos within yolk and amniotic sacs are dissected from the mother. They are then cultured in bottles containing 10 ml male heparinized, heat-inactivated plasma under sterile conditions at 38°C in a specially designed apparatus which insures rotation of bottles.

MAJOR FINDINGS AND PROPOSED COURSE: Embryos of pregnancy day 11 have been grown in culture to 3-4 times their originial size in 24 hours as assessed grossly and by DNA and protein content. In these embryos, major organs, e.g., brain, neural tube, sensory organs, heart and circulatory system, gut, liver, and masculature, apparently differentiate normally.

The rate of rotation of bottles (40 rpm), composition of gasses (5% CO_2, 20% O_2, and balance N_2) and the time and stage of development at which culture should be initiated (3-6 PM of pregnancy day 11) have been standardized for optimal development. Morphological (gross, histological, and EM) and some biochemical parameters (nucleic acid, protein, α-fetoprotein, fetal hemoglobin, embryo specific estrases, etc.) are being developed for evaluation of growth and differentiation. Assessments of the depletion of important nutrients and hormones and the accumulation of excrements during the culture are also being made presently.

Our immediate objectives are (a) to devise methods for culturing embryos at stages of very early organogenesis with continued differentiation and growth by providing specific nutritional and hormonal requirements; and (b) to study effects of environmental chemicals, potential teratogens and carcinogens, on growth and differentiation of embryos *in vitro* utilizing the parameters described.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A significant percent of developmental anomalies in humans is presumably due to exposure to adverse environmental factors. It is also known that most major organ malformations appear during the critical stage of organogenesis. An embryo culture system which parallels normal development during the period of organogenesis under controlled conditions provides opportunities to study cellular and molecular processes associated with the normal and abnormal development. Studies of teratogens and carcinogens with this *in vitro* model will be useful in setting priorities for further screening, and for the study of mechanisms of toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70020-05 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Environmental Agents on Ontogenesis After Parental Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Staples	Research Physiologist	LET NIEHS
OTHER:	P. Nawrot	Visiting Fellow	LET NIEHS
	R. O. Cook	Senior Sanitary Engineer	LEB NIEHS

COOPERATING UNITS (if any)
Laboratory of Environmental Biophysics, NIEHS

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Teratology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The long range purpose of this project is to determine the developmental toxicity and teratogenic potential of selected environmental agents in mammals by application of the test agents to the parent(s) before mating or to the dam during gestation. Work on the chemicals listed previously has been terminated and manuscripts have been published or are being prepared. Studies are being conducted on various types of noise up to 130dB, on microwave, and on solvents. The mouse is used as the experimental animal.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Test agents were given to pregnant females of four species: mouse, rat, hamster, and rabbit by two or more routes, e.g., per os, subcutaneous, intrauterine, during various periods of ontogenesis. Doses were selected on the basis of preliminary study. Those which produced a given pharmacological response (maximum tolerated dose, comparable blood levels, 50% fetal mortality, etc.) were selected. Stresses or disease states that may be elicited before mating were also studied for teratogenic potential either alone or in combination with other environmental components. An attempt was made to compare the sensitivity of various approaches. Qualitative or quantitative differences or similarities in teratogenic response were noted. Resulting offspring were examined for alterations in the incidence of external, visceral, and skeletal changes shortly before term.

MAJOR FINDINGS AND PROPOSED COURSE: Mated mice were exposed to jet engine noise at 130dB continuously for 12 hours per day from days 1 through 6 or from days 6 through 15 of gestation. A decrease in maintenance of pregancy was noted upon comparison to chamber controls or to those left in the animal quarters. Significant differences were not noted between the noise-exposed and control groups in regard to fetal weight or to either incidence or spectrum of malformations seen. Additional mice were then sequentially exposed to 1 of 3 types of noise (jet engine, bells, and sonalert) emitted randomly as controlled by a microprocessor for 25% of each day during the exposure periods identified above. A significant decrease in the percentage of females remaining pregnant was noted in the group exposed to noise from days 1 through 6 of gestation but no increase in the incidence of external, visual, or skeletal malformation was noted. An additional study, in progress, involves exposure to very high frequency noise to which rodents are especially sensitive. Endogenous corticosteroid levels are measured on some mice of each group as an estimate of stress induced.

The solvents selected for study are benzene, toluene and xylene.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Modern man is exposed continuously to a multitude of chemical and infectious insults as well as to physical stresses. Most of these agents have not been tested for teratogenic potential. Through the efforts described the teratogenic potential of selected environmental agents is evaluated in animals. Jet engine and sonalert noise were studied since more than 3500 women of child-bearing age are currently being exposed to this type of noise at up to 130dB in the United States alone.

PUBLICATIONS

Kimmel, C. A., Cook, R. O., and Staples, R. E.: Teratogenic potential of noise in mice and rats. Toxicol. Appl. Pharmacol. 36, 2: 239-246, 1976.

McKinney, J. D., Mauer, R. R., Hass, J. R., and Thomas, R. O.: Possible factors in the drinking water of laboratory animals causing reproductive failure. Proceedings of the Symposium on Identification and Analysis of Organic Pollutants in Waters, First Chemical Congress of North American Continent, Mexico City, Mexico. In L.H. Kieth, (Ed.): Identification and Analysis of Organic Pollutants in Water. Chapter 25, Ann Arbor Science, Ann Arbor, Michigan, pp. 417-432, 1976.

Andrew, F. D., and Staples, R. E.: Developmental Toxicity of Medroxyprogesterone in Rabbits. Teratology 15,1: 25-32, 1977.

Schneider, U. and Maurer, R. R.: Asbestos and Embryonic Development. Teratology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Sequence Complexity of Poly(A)-RNA Isolated from Mouse Embryoid Bodies and Teratomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Harris	Senior Staff Fellow	LET NIEHS
OTHER:	A. B. Silverberg	Staff Associate	LET NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Molecular Embryology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The mouse embryoid body/teratoma system OTT 6050 developed by Leroy Stevens appears to be an ideal system for studying the molecular mechanism of cellular differentiation in a mammalian system. Thus the following studies were carried out. Using oligo(dT)-cellulose chromatography, total poly(A)-containing RNA was isolated from simple embryoid bodies and tumors derived from the embryoid bodies. These RNA samples were characterized with respect to poly(A) content, size on denaturing sucrose gradients, and poly(A) tract length. They also were tested for translation activity in an in vitro lysate system. Complementary DNA was synthesized using reverse transcriptase for both the embryoid body and the teratoma poly(A)-RNA preparations by the RNA-complementary DNA hybridization technique (RNA excess). An evaluation of the sequence complexity of the preparations could be made by computer analysis of the hybridization curves. Three classes of poly(A)-containing RNA could be resolved in both embryoid bodies and teratomas based on their relative abundance in the respective RNA preparations.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Embryoid bodies and teratomas were grown, collected and transferred into 129 Sv-Sl^{J} mouse peritoneal cavities every 2 to 3 weeks. Total nucleic acid extracts were prepared by phenol/chloroform SDS method. Poly(A)-containing RNA was prepared by affinity chromatography using oligo-dT cellulose. Poly(A) content was determined by hybridization with excess ^{3}H-poly(dT) with known standards and determination of amount of hybrid using the S1 nuclease assay. The size of the poly(A)-containing RNA was determined on 70% formamide sucrose gradients by hybridization across the gradient with the ^{3}H poly(dT). By RNaseA and T-1 treatment of the RNA preparations and native polyacrylamide gel electrophoresis with known standards, the length of the A tract was determined. ^{3}H-complementary DNA was synthesized using reverse transcriptase, sized and fractionated on alkaline sucose gradients. Nucleic acid hybridizations were carried out under standard conditions. The hybridization curves were resolved into a number of first order reactions (RNA excess).

MAJOR FINDINGS AND PROPOSED COURSE: The poly(A)-RNA preparation contains 2.5-3% poly(A), a number average size of 1800 to 2100 nucleotides and a poly(A) tract length of 60 nucleotides (NT). The ^{3}H-complementary DNA synthesized was about 350 NT for both preparations, and a cut was taken from the alkaline gradient of material 400-600NT. These ^{3}H-C-DNAs were then used in the hybridization analysis. The embryoid body hybridization curve was resolved by computer into 3 classes of sequences. The first or most abundant class represented 10% of the total sequences and from its rate of hybridization is only one or two sequences (of approximate complexity of 2,000NT). The second or middle class represents 60% of the total sequences and is some 100 different 2,000NT long sequences. Finally, the last or most complex class represents about 25% of the total hybridization and some 3,000 different RNA sequences.

A similar analysis of the teratoma total poly(A)-containing RNA hybridization curve indicated again three major classes of sequences: (1) first class representing 20% and containing 2 different sequences (2) middle class representing 54% and some 300 different sequences and (3) the high complexity class representing some 7,000 different sequences and about 20% of the total sequences expressed.

In the future we hope to look at the overlap in gene expression in the embryoid bodies and teratomas. Also we want to look at total and poly(A)-RNA isolated from the nucleus and the relation of this expressed information and what arrives in the cytoplasm for translation into cell specific proteins. We will also look at these processes in various cell lines grown in tissue culture.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An expanded knowledge of how normal and cancer cells function can hopefully in the future be used to apply to the more practical aspects of curing disease and evaluating the effect of the environmental factors on human health. By quantitating RNA sequence expression (total, nuclear, and cytoplasmic), we

may get a better feel for the information flow from the genetic material. By comparing this genetic information flow from cells in different states of differentiation, we may gain additional insight into the molecular mechanisms underlying gene expressions during differentiation. We will then gain some knowledge into the underlying causes of cancer and environmental damage to various organ systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70047-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Regulation of Gene Expression during Differentiation of Teratocarcinoma Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Carter	Staff Fellow	LET NIEHS
OTHER:	S. E. Harris	Senior Staff Fellow	LET NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Molecular Embryology Group

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline t

These studies are directed toward identification of the subset of nonhistone chromatin proteins which control the expression of specific genes during differentiation of teratocarcinoma cells. A DNA probe complementary to the most abundant class of poly(A)-containing RNA's from embryoid bodies and teratoma tumors will be used to determine the specificity of in vitro RNA transcription templated by either embryoid body chromatin or teratoma chromatin. It has been shown that the 5M urea soluble proteins from chick oviduct chromatin contains putative tissue-specific molecules which control transcription of ovalbumin messenger RNA. The 5M urea soluble proteins of embryoid body and teratoma chromatin will be similarly tested by reconstitution of teratoma chromatin urea soluble proteins to the embryoid body DNA-tightly binding protein complex (the reciprocol experiment will also be done) in order to establish that the analogous activity exists in the 5M urea soluble chromosomal proteins from teratoma or embryoid body chromatin. A 2-dimensional gel electrophoresis of the subset of non histone proteins, conferring transcriptive specificity to chromatin, will be used to analyze radiolabeled nonhistone proteins in order to identify that subset of nonhistones involved in gene regulation.

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolation of chromatin from 2.3 M sucrose, purified nuclei by washing nuclei in buffers of successively lower ionic strength, isoelectric focusing and subsequent SDS gel electrophoresis in a high resolution 2 dimensional gel system, autoradiography of S^{35} methionine labeled nonhistone proteins, reconstitution of chromatin from high salt and urea buffers by direct dialysis to low ionic strength with subsequent removal of urea to physiological conditions, in vitro RNA transcription of various chromatin templates using mercurated nucleotides and subsequent sulfhydrylsepharose chromatography to isolate in vitro synthesized RNA transcripts, RNA-DNA hybridization of in vitro transcripts to probe c-DNA made from most abundant poly(A) RNA sequence classes in teratocarcinoma and embryoid body cells.

MAJOR FINDINGS AND PROPOSED COURSE: The differentiative transition of embryonal carcinoma to endoderm can be studied when well-defined marker gene products are obtained such as the c-DNA to alpha fetoprotein mRNA. We propose to utilize the technique of chromatin reconstitution in order to identify the class of proteins in teratocarcinoma chromatin and teratocarcinoma derived endoderm chromatin which control the expression of the alpha fetoprotein gene in the endodermal derivative.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The teratocarcinoma system has been described as a model system for the study of embryogenesis and neoplasia. A knowledge of the molecular species involved at the level of gene expression during differentiation or transformation would provide a rational basis for understanding those processes involved in preneoplastic transformation or events which interfere with the normal program of differentiation processes at the genomic level. The ability to detect those proteins which are responsible for the alteration of the normal program of differentiation may enable the development of sensitive and inexpensive assays for molecules having teratogenic potential.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70049-01 LET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of In Vitro Translation Systems for Specific Messenger RNA's and for Total Poly(A)-containing RNA Preparations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. B. Silverberg	Staff Associate	LET NIEHS
OTHER:	S. E. Harris	Senior Staff Fellow	LET NIEHS
	D. B. Carter	Staff Fellow	LET NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Toxicology

SECTION

Molecular Embryology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The in vivo mouse embryoid body/teratocarcinoma system is being utilized as a model to study the molecular mechanisms of cellular determination and differentiation. In vitro translation systems (wheat germ S-30 and rabbit reticulocyte lysate) will permit the analysis of the patterns of protein synthesis during the differentiation process from embryoid bodies to teratomas. In vitro translation systems will also allow the characterization, of total poly(A)-containing RNA and cytoplasmic ribosomal-associated poly(A)-containing RNA preparations by analysis of synthesized products. Isolation of specific mRNA's (e.g. Alpha feto proteinmRNA) will permit the analysis of gene transcription with cDNA probes during differentiation and the analysis of precursors of the final protein products.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lysates of wheat germ and rabbit reticulocytes will be used to translate mRNA *in vitro*. O'Farrell gels will be used to analyze the protein products of translation. Scintillation fluorography and radioautography will be used to analyze the protein products synthesized both *in vitro* and *in vivo*.

MAJOR FINDINGS AND PROPOSED COURSE: We have completed a preliminary analysis of the *in vivo* pattern of protein synthesis in embryoid bodies and teratomas. We have determined that the wheat germ translation system has difficulty synthesizing proteins greater than 20,000 mol. wt. We have partially purified alpha-feto protein from rat aminiotic fluid and we have begun the characterization of fetal rat liver total poly(A)-containing mRNA. This research plan will be continued. Plans include production of antibody to rat AFP and isolation of teratoma specific protein called glial fibrillar acidic protein (GFA) and production of GFA antibodies. These antibodies will then be used in the *in vitro* translation system for detection of specific protein synthesis and for purification of these proteins associated with specific messenger RNAs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The research is important to the understanding of the molecular events during differentiation and understanding of the mechanism of gene expression in normal, damaged, and cancerous tissues.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70060-04 LET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. McLachlan	Developmental Toxicologist	LET, NIEHS
OTHER:	K. S. Korach	Staff Fellow	LET, NIEHS
	G. W. Lucier	Head, Devel. Tox. Group	LET, NIEHS
	L. Levy	Research Chemist	EBCB NIEHS
	M. Hogan	Mathmatical Statistician	BB, NIEHS
	R. Faith	Immunologist	EBCB NIEHS
	B. C. Bullock	Prof., Comparative Med.	Bowman-Gray School of Medicine
	M. Metzler	Chemist	Univ. of Wurtzburg
	J. C. Lamb, IV	Pathologist	UNC, Cancer Research Center

COOPERATING UNITS (if any)

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Environmental Biology and Chemistry Branch, NIEHS
Environmental Biometry Branch, NIEHS
Cancer Research Center, UNC
University of Wurzburg, Wurzburg Germany

LAB/BRANCH

Laboratory of Environmental Toxicology

SECTION

Transplacental Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	1.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The long range goals of this project are: (1) to evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring; (2) to investigate the mechanisms involved in the production of subfertility in mammals as a result of their in utero exposure to foreign chemicals; (3) to assess the transplacental carcinogenic potential of these compounds; (4) to study the physiologic disposition and metabolism of these compounds in the pregnant animal and fetus; (5) to study chemico-biological interactions of transplacental toxicants, with special emphasis on structure-activity relationships; (6) to determine if prenatal exposure to environmental agents can alter the cytoplasmic receptors for steroid hormones in reproductive tract tissue; (7) to develop and utilize organ culture systems to study the effects of environmental chemicals on the fetal ovary and reproductive tract in vitro; and (8) to evaluate the above animal models as predictors of human response.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the function status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, quantitity, and morphology of ova obtained from the female reproductive tract, and histopathologic evaluations of the ovary including quantitative determinations of the various follicular classes. Biochemical, physiologic, and morphologic procedures were used which included polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, inverse isotope dilution analysis, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS AND PROPOSED COURSE: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock growth promotor and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring. Continuation of these studies has shown that such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β-estradiol. Differential fetal protein binding between DES and estradiol may help explain these results.

Scanning electron microscopy (SEM) of the genital tract of these females has revealed surface ultrastructural abnormalities which may precede neoplastic changes; SEM may provide a tool for early detection of neoplastic change in the affected tissues. This technique was also used to demonstrate hormone-related changes in genital tract tissue during the estrous cycle and with the onset of puberty. The latter study suggests that hormone responsiveness is manifested earlier in the vagina than in the uterus. These studies are being correlated with observations on the steroid receptors in the affected tissues.

In continuation of studies with male offspring from DES treated mice, their serum testosterone levels were determined; these values fell in the same range as the corresponding control values. However, a high incidence of testicular and sperm abnormalities were detected. Seminal vesicle fluid was also abnormal. The fate of Mullerian duct remnants in DES treated males will be determined and the role of these tissues in prostatic lesions evaluated. These results are pertinent to the development of an animal model to study the reproductive tract lesions in humans whose mothers had been given DES during pregnancy. For example, it was recently reported that azoospermia and abnormal sperm morphology were seen in young men whose mothers were treated with DES during their gestations. In both male and female offspring, attempts will be made to establish biochemical/morphological markers for genital tract lesions.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g. β-dienestrol and ω-hydroxy DES) were identified in the mouse fetus and neonate exposed to ^{14}C-DES. The estrogenic activities of a limited series of DES metabolites and analogs were determined. These studies will be expanded with special emphasis on the biological significance of potentially activated metabolites; such data should aid in generalizations to other classes of estrogenic environmental chemicals. In addition, DES metabolism in target/non target tissues and in cell culture will be evaluated.

Studies in organ culture have shown that DES can alter normal differentiation of the genital tract in vitro. These studies will be continued to evaluate the role of organ/organ and cell/cell interactions in genital tract development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such in utero drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic d mage to the developing oocyte or transplacental carcinogenetic changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PUBLICATIONS

McLachlan, J. A., and Dixon, R. L.: Transplacental toxicity of diethylstilbestrol: A special problem in safety evaluation. In, Advances in Modern Toxicology, Vol. 1, Part 1, New Concepts in Safety Evaluation, ed. M.A. Mehlman, R. E. Shapiro and H. Blumenthal, pp. 423-448, Hemisphere Publishing Corporation, Washington, D.C., 1976.

McLachlan, J. A.: Pernatal exposure to diethylstilbestrol in mice: Toxicological studies. J. Toxicol. Env. Health 2: 527-537, January, 1977.

Lamb. J. C., IV., Newbold, R. R., Stumpf, W. E., and McLachlan, J. A.: The development of the luminal surface of the vagina, cervix and uterus of the mouse. IITRI/SEM, 1977.

McLachlan, J. A. and Dixon, R. L.: Toxicologic comparisons of experimental and clinical exposure to diethylstilbestrol during gestation. In, Advances in Sex Hormone Research, Vol. 3, Regulatory Mechanisms Affecting Gonadal Hormone Action, ed. J. A. Thomas and R. L. Singhal, 309-336, University Park Press, Baltimore, 1977.

McLachlan, J. A.: Male as well as female mice are affected by in utero exposure to diethylstilbestrol. Proceedings of Conference on Women and the Workplace, Society for Occupational and Environmental Health. (in press).

McLachlan, J. A.: Transplacental effects of diethylstilbestrol in mice. Proceedings of the Conference on Perinatal Carcinogenesis, NCI Monograph Series. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of Chemical-Recéptor Interactions in Reproduction and Transplacental Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. S. Korach	Staff Fellow	LET, NIEHS
OTHER:	J. A. McLachlan	Developmental Toxicologist	LET, NIEHS
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	L. Levy	Chemist	EBCB, NIEHS
	M. Metzler	Chemist	Wurzburg, Germany

COOPERATING UNITS (if any)
University of Wurzburg
Biometry Branch, NIEHS
Environmental Biology and Chemistry Branch, NIEHS

LAB/BRANCH
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SECTION
Transplacental Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The main objectives of this project are to determine whether the cancerous lesions produced by prenatal DES injections are caused by the chemical form of DES itself or one of its metabolic products; to determine whether DES is metabolized to biologically and hormonally inactive metabolites; to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins; to investigate some of the biochemical mechanisms which contribute to results of prenatal exposure of hormonally active environmental chemicals in the mouse; to attempt to study these same biochemical effects by tissue culture of the mouse reproductive tract; to determine the molecular locus of transplacental toxicity using structure-function relationships of different environmental chemicals, and to determine a biochemical marker for transplacental toxicity. These objectives are being studied by using refined biochemical techniques of hormone receptors and hormone action. The basic physiological effects on hormone synthesis and hormone levels will be studied using chemical extraction techniques and radioimmunoassays. Experiments to determine the carcinogenic nature of hormonally active environmental chemicals will be studied in vivo and tissue culture.

PROJECT DESCRIPTION

METHODS EMPLOYED: Hormonal effects were studied with receptor binding techniques including saturation binding, competition studies, Scatchard plot analysis, sucrose gradient centrifugation, glucose oxidation/utilization, polyacrylamide gel electrophoresis, dual isotope labeling and protamine sulfate precipitation. Biochemical studies employed spectrophotometric enzyme assays and routine chemical isolation and extraction techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Female offspring given prenatal injections of DES result in two distinct groups: those with hormonally non-responsive uteri and those that are hyperstimulated. To understand why some uteri were not horomonally responsive, we first examined the concentration of estrogen receptors in these animals. Results from our laboratory have shown that those animals in the non-responsive group have significantly lower levels of estrogen receptor than controls. Cytosol receptor concentrations in vaginal tissue were not significantly different from controls. Further experiments with the uterus and vagina from DES exposed mice will be continued to determine which step in the mechanism of hormone action is altered. The difference in cytosol receptor levels could not be explained by differential accumulation of receptors in the nucleus since assays of nuclear receptor in these same tissues showed no differences. Receptor differences in control and DES treated offspring were also found in studies demonstrating the responsiveness of the receptors to estrogen administration. In this case the DES treated animals gave an altered pattern of receptor depletion/replenishment as compared with untreated animals. We are now investigating receptor resynthesis, RNA polymerase activities and glucose oxidation/utilization. In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an *in vivo* bioassay and an *in vitro* receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. The results indicated that certain metabolites were inactive while others still retained a certain amount of hormonal activity. This indicates that the metabolism of DES does not result in complete inactivation of the compound. Receptor and plasma binding activities of various DES analogs will be continued to determine the structural site of chemicals exhibiting hormonal and/or carcinogenic actions. Binding studies with different environmental chemicals are being expanded to allow us to determine their potential hormonal activity in the model. Complete hormone action involves the ability of the hormone to influence resynthesis of its receptor; many of these DES compounds showed this hormonal property. Their ability to demonstrate this property was related to their biological efficacy. Cell and tissue culture systems are being developed with cell lines derived from reproductive tracts. These cultures will then be used for experiments concerning the mechanism of action of these environmental chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The association of in utero DES exposure and reproductive tract cancer in human subjects is well documented. Recent development of a mouse model to study these same effects will allow us to investigate this problem more fully in the laboratory. The objectives of this laboratory are to investigate the role of receptor protein-chemical interactions and the biochemical mechanisms associated with the toxicologic responses observed in the reproductive tract following in utero exposure to hormonally active environmental chemicals.

Since our knowledge of environmental chemical effects in utero on the reproductive system of the offspring is so limited, our studies will increase our recognition of other clinical and biomedical problems which may arise from exposure to environmental compounds. Determining the mechanism by which these chemicals may act may help in the development of safeguards against continued exposure.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70070-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transplacental Pathology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Peters	Visiting Scientist	LET NIEHS
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	R. Dunn	Biological Tech.	LET NIEHS
	E. VanStee	Physiologist	EBCB NIEHS
	R. Faith	Senior Staff Fellow	EBCB NIEHS
	R. Hass	Staff Fellow	EBCB NIEHS
	J. West	Postdoctoral Fellow	UNC
	J. Wagner	Doctor of Vet. Med.	Duke

COOPERATING UNITS (if any)
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Div. of Laboratory of Animal Resources, Duke
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LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Developmental Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project is designed to evaluate the effect of environmental chemicals on offspring of female rats subjected to totigestational exposure. Selected biochemical, histological and behavioral parameters are monitored for transplacental pathology. To date studies on four chemicals have been initiated: methyl-n-butyl ketone, enflurane (ethrane), methylglyoxal, and ethchlorvynol (placidyl). No definite conclusions are possible as yet.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mated female Fischer rats are exposed to selected chemicals throughout gestation by appropriate routes beginning on day 0 of gestation and continuing through day 20. A dose causing minimal overt effects at birth is used. Selected physiologic, biochemical, histological and behavioral observations are made at each of 5 stages of development: newborn, weanling, puberty, 5 months and 2 years of age. The organ systems monitored include: liver, kidney, hematopoietic, sensory, central and peripheral nervous system, reproductive, skeletal muscle, immunological, cardiovascular, etc. Standard techniques and assay procedures are employed.

MAJOR FINDINGS AND PROPOSED COURSE: To date totigestational studies on four chemicals have been initiated.

a. Methyl-n-Butyl Ketone (MBK)

1. Pharmocokinetic Studies. Preliminary data on the absorption, distribution and elimination kinetics of MBK have been obtained using 20 day pregnant rats. Following 10, 60 and 180 min. exposures to 1000 ppm MBK females were sacrificed and fetuses were removed. Various tissues of the dam and pups were removed, weighed and placed in sealed vials and frozen until analyzed. Tissue extracts were then analyzed for MBK and metabolites using gas chromatography. Serum MBK concentration of the dam following 180 min. exposure was found to be 44.9 $\pm$ 7 ug/l. Extracts of liver homogenates contained MBK but quantitation was not possible because of unresolvable contaminating peaks. Extracts of fetal carcasses had measurable amounts of MBK (7.8 $\pm$ .5 µg/l). A mass spectrum analysis of fetal carcass extract identified penitanol, and 3-methyl pentanone in addition to MBK.

2. Totigestational Toxicity of MBK. At 500 ppm administered by inhalation the only effect of MBK noted was a 12 to 24 hour delay in parturition.

At 1000 ppm the exposed dams were smaller than corresponding controls which correlated with a decrease in litter size. While birth weight of treated and control animals was comparable, the growth rate of offspring from treated females was decreased at all stages of development to adulthood. Female offspring of treated females showed retarded sexual maturation as determined by time of vaginal opening. Shock avoidance aquisition and retention were deficient in female offspring but not in males. Female offspring of treated animals were significantly more active when tested on an activity wheel. No other lesions have been detected grossly, histologically or biochemically.

Females exposed to 2000 ppm MBK had a reduced food intake but an elevated water intake relative to controls. The decreased food intake was reflected in a decreased weight gain. Offspring were grossly normal. No further data are available as yet.

b. Methylglyoxal

1. Acute Toxicity. Acute studies of oral toxicity have been performed in order to permit dosage selection for chronic experiments. The LD_{50} was determined in non-pregnant, sperm positive females and in females at day 6, 13 and 20 of gestation. There was a positive correlation between LD_{50} and duration of pregnancy. Thus pregnancy appeared to provide a protective effect against methylglyoxal toxicity. Acute toxicity studies in male and females at birth, weanling and adult demonstrated that newborn animals were more sensitive than adult females which were more sensitive than weanlings. Adult males were more resistant than all other groups.

The surviving pregnant animals were allowed to deliver and their offspring were observed. No gross lesions were observed.

2. Totigestational Toxicity. Seventy-two sperm positive females were divided into 6 groups. Each group was given water, 50, 100, 200, 400 or 800 mg/kg of methylglyoxal throughout gestation. At 800 mg/kg there was a 50% reduction in the number of pregnant females giving birth relative to controls. In the adult, methylglyoxal appeared to stimulate the hematopoetic system causing an increase in packed cell volume and in RBC count. Clinical chemistry determinations revealed that offspring of treated animals had elevated blood glucose, CO_2, BUN and triglyceride with lowered uric acid, bilirubin, Creatinine/BUN ratio and SGOT. An elevated blood glucose, BUN and CO_2 with a reduced uric acid and SGOT were also seen in the adult. Autopsy data showed a reduction in organ to body weight ratios in lung and adrenals in weanling offspring of females given 800 mg/kg of methylglyoxal.

c. Enflurane (Ethrane). Enflurane was administered by inhalation at 0.15% for six hours per day for 21 days starting at day 0 gestation. Serum fluoride was monitored for three consecutive days, day 0, 1 and 2. Extrapolation from these results suggest that a plateau level of approximately 1.7×10^{-5}M would be reached by day 5 or 6. Enflurane treated females delivered a larger percentage of small litters (less than 7 pups) than did controls. Birth weight and subsequent growth rate of offspring of treated females were comparable to controls. A variety of behavioral tests showed no significant differences between young treated and control females. Clinical chemistry tests in weanling pups of treated females revealed an increased creatinine phosphokinase activity and elevated triglyceride level. All other parameters were comparable to control.

d. Ethchlorvynol (Placidyl)

Ethchlorvynol was administered daily by oral intubation throughout gestation at 6 doses ranging from 0 (olive oil) to 80 mg/kg. No alteration in maternal weight or fetal development was apparent. Clinical chemistry determinations in weanling animals revealed an elevated blood glucose and a decrease in phosphorus, creatine phosphokinase, SGOT, SGPT and in ($Na-[CL \pm CO_2]$) balance. No differences in behavior have been observed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human population now has a lifetime exposure to an increasingly large number of chemicals with incompletely defined biological activity. Among the least adequately understood characteristics of many of these chemicals are their potential toxicity to man at his most vulnerable stage of development-- conception to birth. While the more immediate transplacental effects such as abortion or teratogenic changes are relatively well studied among these agents, the often more insidious and potentially more harmful lifetime effects have received little attention. The great difficulty in correctly ascribing gestational origin to lesions in the human population which are subtle or do not become apparent until later in life makes laboratory and epidemiological investigations in this area essential. Hopefully on-going studies will yield data which can be used to identify chemicals in the environment which produce adverse transplacental changes at levels to which the human population is exposed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70080-04 LET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	3	0.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

These studies seek to assess the effects of environmental agents on spermatogenesis. Environmental chemicals are tested, test systems validated and new approaches to toxicity testing proposed: (1) examine germ cell repair and DNA synthesis as indicators for chemical mutagenenesis; (2) utilize an integrated approach to study toxic effects on reproductive function; and (3) investigate the toxication-detoxication of polycylic hydrocarbons in gonadal tissue. To date, selected mutagens, trace metals and Freon 22 have been studied. A new sensitive alkaline elution analysis of germ cell DNA appears to be useful as a predictor of environmental agents which damage DNA. A DNA repair deficient mouse strain has been sought. Biochemical and toxic effects of environmental agents on rodent spermatogenesis are also studied by an integrated approach using spermatogenic "marker" enzymes, plasma FSH, LH, testosterone, histologic changes, and the in vitro perfusion of testis with selected environmental chemicals. These biochemical and toxic effects were correlated to functionality of spermatozoa through serial mating studies. These studies contribute to an improved understanding of mechanism of toxicity, the reliability of laboratory animal toxicity tests for mutagenic and reproductive effects and validity of their extrapolation to humans.

PROJECT DESCRIPTION

METHODS EMPLOYED: In vivo DNA repair was studied by labeling spermatogonial DNA with repeated i.p. administration of tritiated thymidine to 15 to 20 day old rats. Test mutagens were administered and seminiferous tubules were isolated. Spermatogenic cells were isolated from tubules by 0.1% collagenase digestion. Elution profiles defining DNA molecular size were obtained by alkaline elution analysis. In vitro and in vivo DNA repair studies were accomplished by pretreating animals with mutagens, followed by tritiated thymidine in the presence of hydroxyurea. To determine comparative DNA repair synthesis in different mouse strains, the following mouse strains were used: CD-1, RF, LP, SWR, nude homo, and nude hetero.

a. Determination of "marker" spermatogenic cell enzymes. Hyaluronidase (H), sorbitol dehydrogenase (SDH), lactic dehydrogenase-X (LDH-X), alpha-glycerophosphate dehydrogenase (GPDH), malate dehydrogenase (MDH), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were assayed in both isolated tubules and isolated spermatogenic cells using standard methods. Ontogeny of these selected enzymes were studied in relation to chronological age and the morphological development of the spermatogenic cells to identify "Marker" enzymes of particular germ cell types. Specific activities of these selected enzymes were then monitored to predict which germ cell types were affected by environmental chemicals.

b. Determination of FSH, LM, testosterone, arylhydrocarbon hydroxylase, epoxide hydrase and glutathione S-transferase and cytochrome P-450 were measured by standard procedures or slight modifications of these procedures.

c. In vivo assessment of fertility. Fertility studies were conducted on rats exposed to the following compounds: sodium tetrahydroborate, aluminum, and Freon 22.

d. Preparation of interstital cells and spermatogenic cells. Adult rat testes were surgically removed and tunica albuginea removed under a steromicroscope. Seminiferous tubules were carefully teased apart in the cold Spinner culture medium and the tubules were gently separated from the interstitial tissue with forceps. To remove residual interstitial cells, the seminiferous tubules were incubated with 0.1% trypsin per total tubules isolated from 10 testes at 37°C for 15 min. At the end of the incubation tryptic activity was inhibited by 10 ml of fetal calf serum and the entire suspension was filtered through a Kodak lens paper. Tubules were recovered, minced and 0.1% collagenase was added followed by incubation for an additional 30 min. Spermatogenic cells were collected by centrifugation after filtering the cell suspension with seminiferous tubular fragments. Spermatogenic cells were purified by repeated differential centrifugation.

e. In Vitro perfusion of rat testis. Rat testis was quickly exercised and the testicular artery was cannulated with a 80-100 micron glass capillary tube with polyethylene tubing. Oxygenated Kreb-ringer bicarbonate solution containing

3% albumin (BSA fraction 4) was used to perfuse testis at 20 ml $g^{-1}hr^{-1}$. Benzo-(a)pyrene 4, 5-oxide-H^3 at different concentration was perfused and its metabolites, benzo(a)pyrene 4, 5-diol and the glutathione conjugate of the diol were determined by combination of differential solvent extraction, thin layer chromatography and liquid scintillation spectrometric assays. Viability of perfused testis was determined by glucose consumption, concentration of glycolytic intermediates, ATP, PO_2 and PCO_2 of both perfusate and effluent.

MAJOR FINDINGS AND PROPOSED COURSE:

a. DNA repair in spermatogenic cells of several strains of mice and CD-1 rats. Alkaline elution analysis is a new sensitive method for the measurements of DNA single strand breaks and crosslinks in mammalian spermatogenic cells, based on the kinetics of alkaline elution of DNA from cell lysates. During the course of studies with various mutagens, it was found that two types of DNA damage occurred in spermatogenic cells: (1) DNA single strand breaks and (2) DNA inter-and intra-strand cross links and/or cross links between DNA strand and nucleoproteins. This crosslinking interaction stabilized DNA strands and DNA became resistent to elution. Furthermore, DNA reacted with polyfunctional alkylating agents such as busulfan, triethylenemelamine, mitomycin C, nitrosourea; these mutagens were found to be resistant to 500 rad X-radiation, suggesting the stabilization of DNA macromolecule against ionizing radiation. These two types of spermatogenic cell DNA damage can be readily determined and could be used for detecting environmental mutagen at mammalian germ cell level. Furthermore, it appeared that the rate of unscheduled DNA synthesis in spermatogenic cells exposed to MMS was not statistically different but the amount of unscheduled DNA synthesis were different in the following order: Rf>CD-1>Nude homo, hetero, PL, 5WR>LP. Screen for suitable mouse strain will be continued and a DNA repair deficient strain will be tested for dominant lethal assays to determine the extent of mutation sensitivity and to assess its usefulness for a routine in vivo mutagen screen.

b. Integrated approach to study toxic effects of environmental chemicals on male reproductive function. Effects of dietary boron on male reproductive function was studied by an integrated and correlative approach. Male rats were exposed to boron at 0, 500, 1000 and 2000 ppm in their diet for 30 and 60 days. The spermatogenic "Marker" enzymes hyaluronidase, sorbitol dehydrogenase, and LDH-X isoenzymes were significantly reduced prior to reduction in seminiferous tubular diameter and/or testicular weight. In contrast, the specific enzyme activities of glyceraldehyde 3-phosphate dehydrogenase and malate dehydrogenase were significantly elevated above control prior to histologic change. Therefore, spermatogenic "Marker" enzymes could be useful in predicting which spermatogenic cell types are affected. Furthermore boron-induced male germinal aplasia was also associated with significantly elevated plasma FSH while LH and testosterone levels were not significantly affected throughout the experimental period. A selective elevation of FSH suggests Sertoli cell function may be affected by boron while steroidogenic activity of Leydig cells were not affected by boron. Male fertility studies indicated that no dominant lethal effects were observed but boron caused antifertility in male

rats. Further studies are necessary to document whether low concentrations of boron in food and drinking water accumulate to toxic levels in gonads and affect human male reproductive function. Effects of Freon 22 on the male rat reproductive function were also investigated. Spermatogenic and male accessory "Marker" enzymes, plasma FSH, LH and testosterone, complete histology, and male fertility were determined. Preliminary studies indicated that Freon 22 exposed male rats demonstrated reduced weights of both prostatic and coagulating glands.

c. Arylhydrocarbon activating and deactivating enzymes in testicular tissue. Microsomal epoxide hydrase and glutathione S-transferase (GSH-S-T) activities were determined with styrene oxide as substrate in rat testis during postnatal development. The ontogeny of these enzymes were also followed in liver for comparison. Testes of 6-day old rats had high GSH-S-T activities (66 nmole min^{-1} $mg\ protein^{-1}$), about 50% of adult levels. Transferase activity then developed slowly and reached a maximum by 165 days of age. Specific activity of GSH-S-T in testes of 6 day old rats was 3-4 times that of liver. In contrast, epoxide hydrase activities of both liver and testes were very low in prepubertal rats, but they increased dramatically at the onset of puberty and reached maximum activities by 45 days of age. Microsomal and supernatant fractions prepared from rat spermatogenic cells had about twice the epoxide hydrase and GSH-S-T specific activities of the similar fractions from interstitial cells. On the other hand benzo(a)pyrene hydroxylase (AHH) activity and cytochrome P-450 content were at least 2 fold greater in microsomal fractions from interstitial cells than those from spermatogenic cells. Further studies, indicated that testicular AHH was not inducible by 3-methylcholanthrene or phenobarbital pretreatment while liver microsomes were significantly induced. In contrast, HCG (FSH and LH activity of 1 to 1 ratio) treatment to hypophysectomized rats (6 weeks after surgery) induced a 2-3 fold increase of AHH activity in testis but not in the liver. In vitro perfusion of testis with tritium labeled benzopyrene 4, 5-oxide demonstrated that this active metabolite of benzopyrene is rapidly metabolized by EH and GSH-S-T. The rate of metabolism of this active metabolite by testis appeared to follow a first order kinetics and were concentration dependent. GSH-S-T conjugation rate was 10 fold greater than that of EH activity. This difference further demonstrates close similarity to the cell free system. Glycolysis of perfused testes indicated that glucose consumption also followed a first order kinetics and no accumulation of lactate was found. This together with electron micrographic studies suggest that the perfused testis was viable up to 120 min pp. Future studies will continue to screen DNA repair deficient mouse strains. DNA repair deficient mice will be tested for their usefullness in dominant lethal tests as compared to CD-1 mice strains. In vitro and in vivo treated spermatogenic cells will be used to further test and determine the sensitivity of alkaline elution to assess DNA damage induced by various classes of compounds. Future studies will continue to assess the effects of environmental agents on germ cells and will include chronic exposure of environmental agents to rats to simulate real environmental situations in order to extrapolate to human risk. More specifically: (1) investigate the effects of environmental agents on the regulation of differentiation and maturation of germ cells at

a molecular level and further correlate regulatory processes with both ultrastructural abnormalities and functionality of spermatozoa; (2) test selected environmental agents for effects on germ cell development and function; (3) perfuse testis in vitro as a model to study pharmacokinetic parameter of environmental chemicals, chemical activation, deactivation, interaction between cellular macromolecules; (4) evaluate epidemiological data regarding chemically-induced effects on fertility and reproductive tract function.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent times, the mutagenic and carcinogenic potential of environmental chemicals has become a major concern for human health. Consequently, many useful short term mutagenic test systems employing certain bacterial mutagens such as Ames test and, in addition, test systems using mammalian cell lines have become available. The currently available systems to detect mammalian germ cell mutations are the cytogenetic, dominant lethal test and a recessive specific locust assay in mice. Therefore, detection of spermatogenic cell DNA damage can be a useful tool in predicting mutagenic potential of environmental chemicals.

Elution patterns of DNA single strand breaks and stabilization of DNA and/or increased DNA repair synthesis in spermatogenic cells after chemical exposure are found to be useful as mutagenic and toxic indicator of mammalian germ cells. Studies of toxic effects of environmental agents on spermatogenesis and reproductive organs by integrated approach were found to be important in explaining the mechanism of toxic effects. Alteration of spermatogenic "Marker" enzymes was useful in early prediction of specific spermatogenic cell damage. The in vitro cell free system and in vitro perfusion of testis to study chemical activation and deactivation by interstitial and germinal compartments of testis have provided important information with respect to polycyclic hydrocarbon activating and inactivating enzymes in germinal tissues as well as in steroidogenic cells. Furthermore, in vitro perfusion of testis provide a unique model system to study integration of molecular, biochemical and physiological data to pharmacokinetic parameters. This integrated approach to study toxic effects of environmental chemicals on male reproductive organs may serve as an important model for the study of infertility in the human populations.

PUBLICATIONS

Shen, R. S. and Lee, I. P.: Developmental Patterns of Enzymes in Mouse Testis. J. Reproduction and Fertility 48: 301-305, 1976.

Dixon, R. L., Lee, I. P. and Sherins, R. J.: Methods to Asses Reproductive Effects of Environmental Chemicals: Studies of Cadmium and Boron Administered Orally. Environ. Health Perspec. 13: 59-67, 1976.

Lee, I. P., Schmid, B., and Zbinden, G.: A Simplified Method for the Isolation of Sperm Heads from the Caput Epididymidis of Rodents and from Ejaculated Rabbit Seminal Plasma. Exper. Cell Biology 45: 48-59, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70120-06 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism and Binding of Steroids and Xenobiotics in Developing Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.3	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the long-range plan of this project to study steroid and xenobiotic metabolism and binding in target tissues with emphasis on hormonally-active chemicals. We have studied the placental transfer and fetal uptake of chemicals such as the PCBs, PBBs, cadmium, DES, and TCDD. The pharmacokinetics of these chemicals are studied in relation to possible developmental toxicity, emphasizing chemically-induced alterations in the perinatal imprinting of hepatic enzyme activity. The sequence of biochemical events leading to programmed responses in control and neonatally-treated animals are investigated so that predictions might be made regarding structure-activity relationships. The perinatal development of steroid receptors in the liver and brain are evaluated for their influence on the programming process. Results have shown that metabolites of PCBs that are readily hydroxylated accumulate in the fetal intestine following maternal exposures; whereas, concentrations of the biologically-persistent isomers increase markedly in most tissues immediately after birth as a function of milk transfer and redistribution from brown fat.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In most aspects of these studies, timed-bred CD-1 rats are used. For the PCB and PBB perinatal pharmacology studies, ^{14}C-labeled isomers are used. Analogs investigated have been 4-chlorobiphenyl (1-CB), 4,4'-dichlorobiphenyl (2-CB), 3,4-3',4'-tetrachlorobiphenyl (4-CB), 2,3,6,-2',3',6'-hexachlorobiphenyl (6-CB'), 2,4,5-2',4',5'-hexachlorobiphenyl (6-CB), and 2,4,5-2',4',5'-hexabromobiphenyl (6-BB). Metabolites were identified in some tissue samples by thin layer and gas chromatography. Tissues investigated in the PCB and PBB studies from fetal, newborn, and maternal tissues included blood, milk, white fat, brown fat, liver, lung, kidney, brain, male reproductive tract, female reproductive tract, placenta, muscle, and skin. Rats were generally treated orally with the PCBs or PBB on the 18th day of gestation or two weeks prior to mating. By administering the chemicals prior to mating, equilibration is reached between blood and adipose tissue before there is a fetal compartment. The relative contributions of placental transfer and milk secretion to newborn tissue levels are evaluated by foster mother experiments. Attempts are being made to develop a pharmacokinetic model for 6-CB and 6-BB tissue distributions during the perinatal period in collaboration with LEP and BB.

The developmental toxicities of 1-CB, 4-CB, and 6-CB were studied in dose-response experiments by administering the organohalogen 5 times between the 8th and 18th days of gestation.

In animals exposed to hormonally-active xenobiotics during the perinatal period steroid receptors in rat liver cytosol were measured by a variety of assay procedures utilizing dextran-coated charcoal, ammonium sulfate, protamine sulfate, and sucrose gradients. Total and available binding sites are measured. Binding constants and amounts of receptors are determined by Scatchard Plots.

Enzyme assays for UDP glucuronyltransferase (steroid and non-steroid), β-glucuronidase, biphenyl 2- and 4-hydroxylase, and benzpyrene hydroxylase were performed by standard procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Although much is known about the fate of specific PCB isomers in adult animals, little information is available concerning the pharmacology of these isomers in perinatal systems. Therefore, we have studied specific fetal and newborn tissue distribution of several ^{14}C-labeled chlorinated biphenyls. Pregnant rats were administered the labeled PCBs on the 18th day of gestation and levels of radioactivity measured in maternal blood, liver, intestine, fat, skin, and muscle; fetal blood, liver, lung, intestine, kidney, brain, skin, and reproductive tract; and placenta. PCBs that do not accumulate significantly in adipose tissue do accumulate dramatically in fetal intestine. Fetal intestine to blood radioactivity ratios on the 21st day of gestation (3 days after treatment) were as follows; 1-CB (41), 2-CB(22), 6-CB'(18), 4-CB(3), 6-CB(1). Values of 1-CB at the 20th and 19th days of gestation were 20 and 7, respectively. By 5 days after birth, intestine to blood ratios were approximately one for all PCBs. When 1-CB was

administered at the 15th day of gestation, intestinal accumulation still did not occur until the 19th day of gestation, corresponding with the developmental onset of hepatic glucuronyltransferase. Approximately 8% of the fetal intestinal radioactivity following 1-CB treatment was parent compound, 10% 4'-hydroxy-4-chlorobiphenyl and most of the remainder represented conjugates. Our proposed mechanism for accumulation of PCB metabolites in the fetal intestine assumes hydroxylation of the PCB in the maternal compartment. The hydroxylated metabolite passes the placenta and is glucuronidated in the fetal liver; the glucuronide reaches the fetal intestine and is hydrolyzed to the aglycone by β-glucuronidase. The aglycone is reabsorbed and reglucuronidated in the fetal liver, thus reinitiating the cycle.

6-CB, which does not accumulate in the fetal intestine, is secreated in the milk although significant newborn tissue levels of this isomer also result from placental transfer. Newborn skin and fat contained the highest concentrations of 6-CB after gestational and/or milk exposure. Late in gestation, 6-CB appears to accumulate in the brown fat. This adipose tissue is oxidized immediately following birth for the purpose of maintaining body heat and also as an energy source. The depletion of brown fat results in a redistribution of 6-CB to other tissues the first week after birth. Therefore, the pronounced increase in newborn tissue concentrations is a function of 6-CB redistribution from brown fat as well as newborn exposure to this chemical during suckling.

Preliminary studies on 6-BB have demonstrated that the placental transfer rate of this isomer is slower than the rate for 6-CB, but the milk concentrations of 6-BB are significantly greater following equivalent maternal doses.

Studies are in progress to determine the relative rates of placental transfer and milk secretion of 6-CB and 6-BB when the organohalogen is administered to female rats two weeks prior to mating. This protocol allows for equilibration between fat and blood before there is a fetal compartment.

PCB administration to pregnant rats resulted in variable degrees of hepatic enzyme induction during perinatal development as measured by UDP glucuronyltransferase (UDPGT) activity. For example; 1-CB had no effect on prenatal or postnatal activities; 4-CB induced UDPGT 3-4 fold at 6 and 20 days after birth but no effects were evident in 55-day old animals; enzyme activities in the 6-CB groups were the same as controls in prepubertal rats whereas a feminization of activity (male rat liver UDPGT activity is greater than female activity in controls but male and female activities were similar to the 6-CB group) occurred in 55-day old male rats exposed to 6-CB during the perinatal period.

Future studies will concentrate on the elucidation of factors regulating placental transfer and milk secretion of the PCBs and PBBs. A pharmacokinetic model will be derived for 6-CB and 6-BB specific tissue concentrations resulting from placental passage and newborn exposure during suckling. The pharmacokinetic studies will be correlated with a developmental toxicity study emphasizing organ functions of liver, intestine, skin, and reproductive tract.

Cytosolic and nuclear steroid receptors will be studied in liver and brain in an attempt to determine the role of hormone action in the neonatal imprinting of hepatic enzymes by hormonally-active chemicals such as zeranol, DES, and the PCBs. A wide variety of hepatic enzymes representative of specific subcellular organelles will be evaluated for estrogen and androgen responsiveness in control, castrated, ovariectomized, and hyphophysectomized animals to determine the role of the gonads and pituitary in hepatic enzyme responsiveness to steroids.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pharmacodynamic properties of environmental agents in perinatal systems can be an extremely important determinant of target organ sensitivity to various forms of developmental toxicity. Susceptibility to classes of chemicals during the period of development and differentiation is often determined by metabolism (toxication or intoxication), transport, and binding of the parent compound and for toxicologically-important metabolites. Placental transfer rates of chemicals as well as the partition into milk are of particular interest for perinatal pharmacology studies. We have concentrated our efforts on organochlorine agents that cause or have the potential to cause serious environmental health hazards such as the PCBs, PBBs, TCDD, heavy metals such as cadmium and mercury, and synthetic steroids such as diethylstilbestrol. The perinatal pharmacology of hormonally-active xenobiotics is emphasized due to the obvious importance of hormonal control to differentiation and development. The experiments are designed so that structure-activity relationships are elucidated for the perinatal pharmacology of groups of chemicals. For example, the pharmacology of the PCBs, including placental transfer and milk secretion, was studied in relation to positions and number of chlorine substitutions and the pharmacokinetics of 6-CB and 6-BB were directly compared. The perinatal pharmacology studies provide direction for subsequent target-organ toxicity studies. Considerable research is in progress regarding the characterization of steroid metabolism and steroid receptors. However, relatively little attention is accorded the role that steroid metabolism and steroid binding play in developmental and reproductive toxicity. Our studies attempt to characterize receptors and enzyme systems as "markers" for certain types of carcinogenesis and teratogenesis. Of particular interest is the ability of xenobiotics to alter neonatal "programming" of steroid metabolism and the role that such alterations might have in developmental toxicity.

PUBLICATIONS

Lucier, G. W., Lee, I. P., Dixon, R. L.: Effects of environmental agents on male reproduction. The Testes. Vol. IV., Academic Press (in press).

Lucier, G. W.: Developmental aspects of xenobiotic biotransformation. Environ. Health Persp. 18: (in press).

Lucier, G. W., Sonawane, B. R., and McDaniel, O. S.: Glucuronidation and deglucuronidation reactions in hepatic and extrahepatic tissues during perinatal development. Drug Metab. Dispos. (in press).

Lucier, G. W. and McDaniel, O. S.: Differentiation between steroid and non-steroid glucuronidation. J. Ster. Biochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70131-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of Intestinal Functions: Normal and Toxic Responses

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.3	1.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline)

It is the long-range purpose of this project to study the perinatal development of the functioning small intestine in normal and environmentally-, nutritionally- and hormonally-stressed animals. The topics of present interest which relate to the development of the normal functioning small intestine are: (1) effect of prenatal exposure to hydrocortisone, hydrazine and dimethylhydrazine (DMH) on the development of enzymic indicators of membrane integrity and function, e.g., alkaline phosphatase, acid phosphatase, Na,K-ATPase, lactase and sucrase; (2) role of cell specificity in the susceptibility to the TCDD induction of UDP-glucuronyl transferase in intestinal crypt and tip cells in vivo and in vitro; (3) determination of the characteristic isozyme profile for β-glucuronidase during development and after DMH treatment as a possible indicator of toxicity and a precancerous state; (4) examination of intestinal membrane and serum marker enzyme development after prenatal exposure to polychlorinated biphenyl structural analogues; (5) relative contribution of the tip and crypt cells to energy metabolism of these cells; (6) mechanism of As-induced toxicity of mitochondrial function in normal and diabetic animals and (7) application of the isolated cells technique to metabolic and toxicologic problems.

PROJECT DESCRIPTION

METHODS EMPLOYED: The preparation of subcellular fractions and mucosal scrapings at the various developmental stages and the measurement of enzymes, metabolites and substances were performed by adaptation of standard procedures. Endpoint assays were developed for the intestinal membrane and function marker enzymes, e.g., sucrase, lactase, alkaline phosphatase, acid phosphatase and Na, K-ATPase. The enzymes were used to monitor the development of intestinal membranes and their function in animals exposed prenatally (11th day of gestation) to compounds such as hydrocortizone, hydrazine and hydrazine derivatives.

β-glucuronidase (βG) was assayed at pH 4.5 using p-nitrophenylglucuronic acid as substrate. The levels of βG were monitored in intestinal whole homogenates during the development of the Fischer rat. The reported chromatographic methods for separation of hepatic βG isozymes were used as an initial reference point for studying the intestinal βG isozyme profile. The identification of separate intestinal βG isozymes from fetal, neonatal, adult, normal and DMH-treated tissue were obtained by polyacrylamide gel electrophoresis.

The TCDD induction of UDP-glucuronyltrasferase in tip and crypt cells isolated from the duodenum of female rats was monitored with the change in time after intubation, of 16μg TCDD/kg. The rats were fasted overnight before treatment and then allowed to feed ad libitum thereafter. The isolated cell preparations were monitored throughout for cellular integrity as indicated by trypan blue dye exclusion, cell counts and morphologic examination by electron microscopy and cell separation as indicated by the cell marker enzymes alkaline phosphatase, acid phosphatase, βG, and thymidine kinase. The induction process in intestinal tip and crypt cells was also evaluated by in vitro exposure to TCDD and monitoring the enzymes after 1, 2 and 3 days in rotating suspension cell culture.

The effects of prenatal exposure to 4-monochlorobiphenyl, 3,4-3',4'-tetrachlorobiphenyl and 2,4,5-2',4',5'-hexachlorobiphenyl were evaluated at the -1 day prenatal and +6, +20 and +56 day postnatal stages by measuring intestinal alkaline phosphatase, acid phosphatase and monoamine oxidase levels in the dams and pups. Serum levels of alkaline phosphatase, β-hydroxybutyrate dehydrogenase and sorbitol dehydrogenase were also used as indicators of tissue damage. Tissue was also examined at the light level for histological alterations.

The relative contribution of the isolated crypt and tip cells to intestinal energy metabolism was evaluated by glucose utilization and lactate/pyruvate output. The cell integrity was investigated by measuring the release of lactate dehydrogenase, NADH-L-glycerol 3-phosphate dehydrogenase, citrate synthase and β-hydroxybutyrate dehydrogenase (two cytosolic and two mitochondrial enzymes). After the subcellular fractionation of the cell types was verified using these same marker enzymes, the distribution and levels of the NADH substrate cycle enzymes were measured. The effect of specific inhibitors and inducers of the substrate cycle enzymes on the lactate and pyruvate output were evaluated.

The pyruvate dehydrogenase assay for determining both basal and total enzymes levels was utilized to measure the adult rat liver and intestinal activities in animals given a prolonged oral exposure to sodium arsenate (As^{+5}). The mitochondrial anion exchange techniques originally developed for rat liver were adapted to hamster intestine. The kinetic parameters for metabolically important anions, e.g., phosphate, malate, succinate and citrate, were determined for intestinal mitochondria as well as for liver mitochondria As-treated animals. The methodology for studying mitochondrial pyruvate transport is also being established.

MAJOR FINDINGS AND PROPOSED COURSE: The observed developmental pattern of pyruvate dehydrogenase indicated a 3-fold increase in the basal to total activity ratio at the perinatal period. Other mitochondrial enzymes such as citrate synthase and β-hydroxybutyrate dehydrogenase did not change at this stage. This change in ratio was correlated with an increase in the redox state of the mitochondria as calculated from the acetoacetate to β-hydroxybutyrate ratio. This increase in activities of PDH was reversed in the 1-day old animals by exposure to N_2 and, agrees with the concomitant decrease in the mitochondrial redox state.

Prenatal exposure to hydrocortizone, hydrazine and dimethylhydrazine resulted in little change in the normal development of intestinal lactase and sucrase. The greatest effect of hydrazine and dimethylhydrazine was observed on the development of intestinal Na,K-ATPase which doubled during postnatal development. However, there were smaller increases in alkaline phosphatase and acid phosphatase in animals treated with hydrazine and dimethylhydrazine, respectively.

The distribution of the marker enzymes along with electron micrographs establish the separation and integrity of the tip and crypt cells. Initially, there was a 5-fold difference in the activities of alkaline phosphatase and thymidine kinase which were employed as markers for the tip and crypt cell types, respectively. Acid phosphatase and β-glucuronidase were distributed evenly in these cell preparations. The UDP-glucuronyltransferase activity was twice as high in the tip cells as the crypt cells. A single dose of TCDD, 16 µg/kg given by gavage, altered only the UDP-glucuronyltransferase levels. The time course indicated that this initial increase was in the crypt cells and that the later increase which appeared in the tip cells agreed with the transit time of the epithelial cells up the villous wall.

The subcellular distribution of β-glucuronidase was examined in the liver and small intestine from adult Fischer rats. Approximately 90% of the total liver activity was located in the lysosomal and microsomal fractions. In contrast, approximately 70% of the intestinal β-glucuronidase from liver lysosomes and the intestinal cytosol was further characterized to reveal two distinct peaks of β-glucuronidase activity in liver and one in intestine. β-glucuronidase was also studied in the developing intestine of the Fischer rat. A pronounced peak of β-glucuronidase activity was evident during the second week after birth. Specific activities in the newborn were approximately three times

greater than adult activities. The isozyme profile from neonatal, normal adult and DMH-treated adult intestine indicated distinct isozyme profiles of β-glucuronidase activity for each situation.

There was no systematic pattern of alteration in the membrane marker enzymes in either the dam or pup intestine after exposure to the 4-mono, 3,4-3',4-tetra and 2,4,5-2',4',5-hexachlorobiphenyls. Of the serum enzymes studied developmently, only the sorbitol dehydrogenase and β-hydroxybutyrate dehydrogenase in the dams serum showed a marked increase after exposure to the 4-monochlorobiphenyl isomer.

We plan to concentrate our efforts in fiscal year 1978 on follow-up studies based on several of our current projects. In particular, we are interested in studying (1) various aspects of energy metabolism in isolated intestinal cells, (2) effects of prolonged arsenic exposure on mitochondrial anion transport, on alloxan-diabetes on pyruvate metabolism via pyruvate oxidation and citrate utilization for fatty acid synthesis, (3) the time course of TCDD induction of intestinal cell UDP-glucuronyltransferase in vitro, (4) β-glucuronidase isozymes on SDS-PAGE and via histochemical techniques, (5) intestinal enzyme induction, metabolism and/or transport utilizing the rotating suspension cell culture system, (6) the programming of rat liver mitochondrial enzymes, intestinal enzymes and possibly intestinal binding proteins and (7) the effects of citrate and phthalate esters on mitochondrial anion transport.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the past, perinatal development studies of the small intestine have dealt primarily with hydrolytic enzymes, the brush border, and the absorption of specific nutrients. Our emphasis has been on the development of the metabolic energy producing processes required for the active transport of nutrients and also the effect of various bioactive environmental compounds on the normal developmental patterns of this target organ. Currently, our laboratory is using a variety of biochemical and morphological techniques to study several aspects of the development of normal intestinal function, mechanisms of intestinal toxicity and indicators for response to toxic insults. (1) In vitro studies have shown that PDH regulation is sensitive to changes in the concentrations of a number of physiological metabolites. Likewise, our recent studies show that intestinal tissue taken from animals during the perinatal period contains PDH activity which is sensitive to in vivo changes in redox state. The conversion of PDH to its active form, immediately after birth and after exposure to hypoxia, is in the direction predicted by the concomitant change in the calculated mitochondrial free NAD+:NADH ratio. (2) Our results from the study of prolonged oral exposure to arsenic indicate changes in the mitochondrial utilization of pyruvate. The arsenic effect on PDH activity after three weeks of exposure is characterized by a decrease in basal activity, a decrease in total activity and an increase in the relative case of activation. This last effect may be indicative of a stimulation of the phosphatase, an inhibition of the kinase and/or an effect directly on PDH which is not lipoic-acid dependent. These observed effects on mitochondrial metabolism support previous in vitro mitochondrial studies and also indicate that the mechanism appears to be more

complex than previously suggested. (3) The initial studies on the TCDD induction of UDP-glucuronyltransferase in isolated crypt cells suggest a model system for studying the role of cell turnover and specificity in the susceptibility to the induction process. (4) The mammalian enzyme β-glucuronidase is involved in the enterohepatic detoxification-intoxication process of a wide variety of chemicals. The normal function of this enzyme in the intestine is not yet well understood. Since fetal and newborn tissues often exhibit enzyme activities similar to tumor tissue, their intestinal isozyme profiles are compared to adult intestinal profiles following exposure to a colon carcinogen, dimethylhydrazine. These studies may provide an indicator, specific isozyme for identifying precancerous or neoplastic tissues. (5) An intestinal epithelial culture system formed by the isolation of tip and crypt cells is potentially useful in the study of in vitro differentiation and intestinal toxicity by bioactive environmental compounds and hormones.

PUBLICATIONS

Schiller, C. M., Editor, An Analysis of the scientists' responsibilities in the acquisition and utilization of human tissue for in vitro research. In Vitro 13: July, 1977 (proceedings issue).

Fowler, B. A., Woods, J. S., and Schiller, C. M.: The ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ. Health Perspec. 19: August, 1977.

Schiller, C. M., Fowler, B. A., and Woods, J. S.: Arsenic effects on pyruvate dehydrogenase activation. Environ. Health Perspec. 19: August, 1977.

Schiller, C. M.: Pyruvate dehydrogenase activity in hamster small intestine during development. Biochem. J. 164: 1977.

Schiller, C. M., and Walden, R.: Development of substrate cycle enzymes in the hamster small intestine. Develop. Biol. (in press).

Schiller, C. M., Huang, T. I. and Heizer, W. D.: Isolation and characterization of four peptide hydrolases from the cytosol rat intestinal mucosa. Gastroenterology 72: 93-100, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70135-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Effect of Hormones and Xenobiotics on the Regulation of Perinatal Enzyme Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
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SECTION
Developmental Toxicology Group

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Our research efforts are directed toward investigating the effect and mode of action of hormones and xenobiotics on the regulation of enzyme patterns during perinatal development. Particular emphasis has been placed on the postnatal development of hepatic histidase in the rat following gestational exposure to diethylstilbestrol (DES) and other hormonally-active xenobiotics. Histidase of the rat undergoes a complex postnatal developmental course, in which its catalytic activity appears shortly following parturition and subsequently rises in a multiphasic sex specific fashion, resulting in enzyme levels which are considerably higher in the adult female than the male. Recent experiments demonstrate that prenatal exposure to DES results in no change of hepatic histidase activities in immature male and female offspring and adult male offspring. However, histidase activities of adult female rats were decreased by 30% and approached activities of adult males. Thus, DES elicits a prenatal programmed response of hepatic histidase. Since sexual differences in hepatic metabolism are believed to be regulated by the hypothalamic-pituitary axis, we suggest that DES affects the developing brain and thus metabolic potential in mature animals.

PROJECT DESCRIPTION

METHODS EMPLOYED: Histidase is conveniently assayed by measuring the conversion of the amino acid, histidine, to urocanic acid. Date bred Sprague Dawley CD-1 rats were exogenously administered the following compounds: DES, polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-p-dioxin, Zeranol, 17β-Estradiol, Testosterone propionate given either subcutaneously using propylene glycol as vehicle or orally using cotton seed oil as vehicle. The animals were then sacrificed by decapitation and allowed to bleed; livers were rapidly removed. Twenty-five percent liver homogenates were prepared, spun down at 105,000g and the soluble portion was assayed for enzyme activity. Bilateral ovariectomies were performed on 42 day old females using methoxyflurane as the inhalation anesthetic.

MAJOR FINDINGS AND PROPOSED COURSE: Hepatic histidase of the rat undergoes a complex postnatal developmental course: the enzyme is detectable shortly after parturition, rises slightly thereafter, maintaining relatively low titers for the first two to three postnatal weeks; then a marked increase in enzymatic activity is manifested in both sexes throughout the adolescent period; this secondary rise, being of greater magnitude in the female than the male, attains levels that are approximately two-fold higher in adult females than adult males. This developmental course is the result of concerted actions of several hormones and nutritional factors acting at specific developmental stages: Glucagon (via cyclic AMP), glucocorticoids, estrogen and amino acids serve as histidase inducers; growth hormone, ACTH and thyroxine as enzyme suppressors. The pituitary is the source of factors(s) which are both suppressive to the postnatal development and to the maintenance of adult steady state levels of hepatic histidase.

We have demonstrated that DES effects rat liver histidase in a manner similar to 17β-Estradiol (E_2). Daily subcutaneous injections of 100 mg/kg b.w. E_2 or DES for one week to 28 day old male rats elevated hepatic histidase activities to 177 and 175%, respectively, of control values. Adult ovariectomized rats receiving E_2 or DES on the same schedule exhibited histidase activities that were 149 and 141%, respectively, of controls.

Oral administration of DES (100μg/kg b.w.) to pregnant rats at the 15th day of gestation had no effect on histidase activities in immature male and female offspring and on adult male offspring. However, histidase activities of these intact adult female rats were decreased by 30% and approached activities of adult males. Exogenously administered E_2 to these intact prenatally DES treated adult females and to the ovariectomized same resulted in elevated histidase activities that are similar to normal intact females.

We therefore conclude that DES administered during gestation is responsible for a masculinization of hepatic histidase activity in the adult female rat. Since no effect is seen in young animals (up through weaning), and since an effect is seen in pubertal animals through adulthood, DES appears to elicit

a programming effect by masculinizing hepatic histidase activity levels in adult females. This response may be reversed by the exogenous administration of estrogen.

Preliminary observations indicate that some of the other hepatic enzymes may also be affected. Prenatal administration of 3,3',4,4'-tetrachlorobiphenyl resulted in increased levels of cytosolic histidase and of microsomal UDP-glucuronytransferase in prepubertal rats. Feminization of UDP glucuronyltransferase in male rats and masculinization of testosterone Δ^4-5a-reductase (higher enzyme levels in normal females than males) were observed in male and female offspring prenatally exposed to 2,2',4,4',5,5'-hexachlorobiphenyl. These results indicate that hormonally active xenobiotics administered during gestation are capable of altering the normal sequence of postnatal development and may elicit a programmed effect.

We are currently expanding our investigation of "imprinting" or "enzyme programming" by investigating the postnatal effect of androgens and DES on hepatic metabolism. Our studies will include the measurement of not only the amino acid metabolizing enzyme, histidase, but also some drug metabolizing enzymes as well (cytosolic drug metabolizing systems and microsomal steroid metabolizing enzymes) in animals that have been prenatally or postnatally exposed to steroids and xenobiotics. Since a pituitary requirement is necessary for complete and normal expression of the developmental pattern of these enzymes we are initiating in vitro studies to complement our present ongoing in vivo investigations. Using a hepatoma cell line we hope to show that we can get positive modulation of histidase activity with estrogen when a pituitary extract is added or when a pituitary cell line is co-cultured. Likewise we want to implant a pituitary into a hypophysectomized animal and conclusively show that pituitary factor(s) are involved in mediating histidase activity. Measurements of circulating gonadotrophins, pituitary hormones and specific receptors should also aid in understanding these mechanisms. In conjunction with these studies we hope to look at protein profiles as markers for developmental toxicity of hormonally-active chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the dominant biochemical aspects of the orderly development of an organism from fertilization to maturity is the continuous progression of enzyme protein formation ultimately resulting in specific enzymatic patterns which constitute and determine the metabolic machinery characteristic of each mature differentiated tissue. The postnatal development of the hepatic mammalian enzyme, histidase is of particular interest in that this enzyme is known to undergo a polyphasic postnatal developmental course in the rat. It is the purpose of our studies to further our understanding of what factors are responsible for this complex postnatal developmental pattern. In addition to studying the normal expression of this enzyme we want to see if xenobiotics can alter the normal expression and discover, if so, how this is accomplished. It is quiet obvious that in our complex society and environment the fetus is ultimately exposed to numerous factors that can have a deleterious effect on the developing offspring. The study reported above is an example of alteration of enzyme expression that may be a marker for abnormal differentiation and

development. A similar effect on enzyme protein complement may result in an altered capacity to metabolize potential teratogens, carcinogens, or drugs and, therefore, allow humans to be more susceptible to biochemical insult. An understanding of these biochemical mechanisms should allow us to make predictions on chemicals that might alter the normal sequence of programming.

PUBLICATIONS

Lamartiniere, C. A. and Feigelson, M.: Effects of Estrogen, Glucocorticoid, Glucagon and Adenosine 3': 5' monophosphate on the Catalytic Activity, amount and rate of de novo synthesis of hepatic histidase. J. Biol. Chem. (in press).

Lamartiniere, C. A., Bridges, E. and Lucier, G. W.: Fetal programming of hepatic histidase by diethylstilbestrol. Red. Proc. 36, 927, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70138-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Effects of Environmental Factors on Programming Steroid Metabolizing Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the intention of this work to examine the phenomenon of programming (imprinting) steroid metabolizing enzymes in the rat in an effort to determine the role of the endocrine system in this process. Initial studies examine the ontogenesis of selected steroid metabolizing enzymes (i.e., 5 α-reductase and 16 α-hydroxylase), noting in particular the programmed divergence in these enzyme activities in the male and female after puberty. Studies will then be performed to determine whether exposure to various hormonally-active xenobiotics can alter the normal process of hepatic and gonadal enzyme programming. The critical period of development will be determined by exposing animals to the xenobiotics either prenatally or postnatally. Particular attention will be given to the enzyme 5α-reductase, which has a dual localization, i.e. in the endoplasmic reticulum and the nucleus. The programming studies on this enzyme are conducted in both subcellular fractions to determine if enzyme localization alters the sensitivity to the programming process. The hormonally-active agents of interest include DES, various PCB isomers, zearanol, and the normal endogenous steroids.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: These studies examined the effects of hormonally-active environmental agents on steroid metabolizing enzyme activity. Specific chemicals included in the studies are DES, zearanol, selected PCB isomers and certain steroid hormones. Environmental agents were administered prenatally (p.o. on days 14 and 18 of gestation to pregnant rats) or postnatally (p.o., day 2 or 7) to newborn rats. Following treatment enzyme activities were studied at various ages of the rat to determine at what age maximum enzyme changes occurred. Animals were castrated at various ages after birth (e.g. 1d, 7d, 27d) to determine the influence of the gonads on enzyme programming.

Developmental patterns of 5α-reductase and 16α-hydroxylase were determined using whole tissue homogenates of the rat liver or testes. Enzyme activities were determined using radioactive substrates; subsequent radioactive metabolites were separated by thin layer chromatography and quantitated by liquid scintillation counting.

Subcellular tissue fractions were obtained using standard procedures. Purified liver nuclear preparations were prepared by the method of Gustafsson and Pousette (Biochem. 13: 875, 1974), using discontinuous sucrose gradients.

MAJOR FINDINGS AND PROPOSED COURSE: Male and female rat hepatic steroid metabolizing enzymes (i.e. 5α-reductase and 16α-hydroxylase) have divergent developmental patterns at puberty. Until about 31 days of age, 5α-reductase activity is approximately the same in male and female rat liver; beyond 31 days of age, female hepatic 5α-reductase activity rises dramatically, while male hepatic 5α-reductase activity remains at prepubertal levels. By 59 days, female 5α-reductase specific activity is more than 10-fold greater than male 5α-reductase activity. Ovariectomy of the female at 27 days of age produces a lower more masculine level of 5α-reductase activity in adults than in intact age-matched controls. Castration of the male rat at 27 days of age allows a more feminine type of 5α-reductase activity to develop at later ages than in the intact male (i.e. 5α-reductase is higher).

In contrast to the developmental pattern of 5α-reductase, sexual differences in 16α-hydroxylase activity are reversed and appear at a slightly older age. Specifically, around 45 days the male levels of 16α-hydroxylase activity begins to rise while female 16α-hydroxylase activity declines. As observed with 5α-reductase, removal of the gonads at day 27 causes the 16α-hydroxylase activity of the male rats to become more feminine (e.g. lower). There is little change in 16α-hydroxylase activity in the female following ovariectomy.

Prenatal exposure of rats to DES (500 μg/kg on days 14 and 18 of gestation, p.o.) produces an apparent masculinization of both 5α-reductase (at 45 days) and 16α-hydroxylase (at 66 days) in the female liver, and further masculinizes 5α-reductase in the male liver (days 59 and 66). The incomplete nature of the observed masculinization may be due to the time of exposure to DES; exposure may not have occurred during the "critical period" of development of normal hepatic enzyme programming. Further studies will examine the effects of DES exposure at different ages on the normal development of steroid-metabolizing enzymes.

In this regard, a preliminary study has shown that neonatal exposure (day 7) to DES may exert a more complete "imprinting" of hepatic enzyme activity than does prenatal exposure. Specifically, 10μg DES (p.o.) given to intact female rats on day 7 produces a 50% decrease in 5α-reductase activity by day 49 (i.e. masculinization) as compared to 49 day old female control levels; 16α-hydroxylase activity is unaffected. This age has been reported in the literature to be important with respect to sexual differentiation of the rat brain.

In animals exposed prenatally (5 doses between gestation days 8-18) to various PCB isomers, some masculinization of both male and female hepatic enzyme activities occurs. Specifically, 2,4,5-2',4',5'-hexachlorobiphenyl was the most effective of the three isomers examined. It produces a decreased level of 5α-reductase in the female liver (at days -1, +6 and +20), and in the male liver (days +20 and +55). An increased level of 16α-hydroxylase activity was observed in female liver (days +6, +20) and in the male liver (days +20 and +55). 3,4,3',4'-Tetrachlorobiphenyl was less effective; 5α-reductase was diminished in female liver at -1 and +6 days, and at +6 and +20 days in the male liver. The 4-monochlorobiphenyl was the least active compound, producing only a slight decrease in 5α-reductase activity in the male liver at 55 days of age.

Future studies will attempt to further elucidate the mechanism by which programming of steroid metabolizing enzymes occurs. The role of the endocrine system in this process will be further investigated through the use of various animal surgical procedures. For example, examination of enzyme activities in castrated, hypophysectomized, or castrated-hypophysectomized animals, will help us to determine the importance of an intact hypothalamo-pituitary-gonadal axis, and which element of this axis is the most important. A comparison of the effects of xenobiotics on various enzyme systems could enable us to determine whether more than one factor is operating in the programming process. In addition, use of an HTC tissue culture system will enable us to determine the role of various factors, including pituitary extract, LH, FSH or prolactin, in the regulation of enzyme activity. A more thorough characterization of the factors which are responsible for sexual differentiation of hepatic steroid metabolizing enzyme activity, and which of these factors are altered by exposure to xenobiotics during the critical period of development, is necessary in order to determine which parameters should be measured as predictive indicators of developmental toxicology.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent reports in the literature have demonstrated that neonatal testosterone administration is capable of altering adult hepatic steroid metabolizing enzyme activities. It is also known that the administration of various hormones during the first week of life can interfere with the normal sexual differentiation of the rat brain. With these facts in mind, it becomes important to recognize the possible consequences which may result following exposure to a hormonally-active environmental agent during a critical phase of development. Such an exposure could alter the normal processes which program steroid metabolizing enzyme systems or sexual differentiation of the brain, with permanent effects on the exposed animal. These studies attempt to characterize how xenobiotics affect normal neonatal hepatic or testicular enzyme programming

mechanisms, to define what role such alterations play in developmental toxicity, and to determine whether such a system can be used as a model for predicting development toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70140-05 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Factors Affecting the Regulation and Metabolism of Urine and Hemoproteins in Mammalian Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

These studies are designed to investigate the biosynthesis, utilization and metabolism of heme, and the regulation of hemoprotein function in mammalian tissues. Particular emphasis will be placed upon understanding factors affecting the regulation of heme and hemoprotein levels at different stages of development and during chronic disease processes. Elucidation of alterations in heme biosynthetic parameters incurred by exposure to hematotoxic environmental agents will be of special interest. Substances under current investigation include various trace metals and the chlorinated hydrocarbon, TCDD. A specific mechanism for the role of heme in the regulation of cytochrome oxidase in fetal liver has been proposed. Current studies suggest that heme may also limit the synthesis of microsomal cytochrome P-450 in mammalian liver under circumstances wherein heme biosynthesis is chronically impaired. A model system in which hepatic heme biosynthesis and mixed function oxidase activity are chronically impaired as result of exposure to the hepatocarcinogen ethionine is being developed in order to assess the effects of impaired heme biosynthesis on microsomal hemoprotein function resulting from exposure to a variety of environmental hematotoxic agents.

PROJECT DESCRIPTION

METHODS EMPLOYED: Studies will be conducted using rats and mice as the experimental animals. Agents for acute studies will be administered via appropriate routes. In chronic toxicity studies, animals will be treated with substances administered via the drinking water or mixed with the diet. Agents will be administered to fetal animals via IP or IV injection of the mother. Data will be analyzed using commonly accepted statistical methods.

Various approaches will be utilized to assess the functional relationship between heme biosynthetic capability and hemoprotein synthesis and activity in mammalian tissues. These include (1) assessment of the time and dose-related effects of agents, known to specifically inhibit or otherwise alter heme biosynthesis and degradation, on the synthesis and functional activation of mitochondrial and microsomal cytochromes in mammalian tissues in vivo; (2) studies of the effects of heme on the turnover of tissue cytochromes following specific inhibition of ribosomal or mitochondrial protein synthesis in vivo; and (3) cytochrome recombination studies in vitro performed to assess the role of heme in the synthesis and functional (re) activation of holocytochromes from induced apoproteins following acute and chronic impairment of heme biosynthesis in various tissues. Rates of protein and heme biosynthesis will be assessed using pulse-labeling techniques with appropriate radioisotopes. In the assessment of transplacental and chronic toxicity, biochemical and morphological studies will be performed to determine the effects of prenatal or prolonged exposure to various hematotoxic agents on the growth and function of tissue organelles and hemoproteins. Development of tests for predicting hematotoxicity in animals as well as human populations will be based on identification of early changes in specific patterns of excreted or circulating heme precursors associated with prolonged exposure to selected environmental agents.

MAJOR FINDINGS AND PROPOSED COURSE: Results of recent studies indicate that heme plays an important role in the regulation and functional activation of cytochrome oxidase in fetal mammalian liver. This action appears to be mediated through stimulation of the synthesis of the apoprotein of cytochrome oxidase and facilitation of the assembly of this apoprotein and heme moities into a functional cytochrome. In contrast, heme has not been shown to regulate either cytochrome oxidase or P-450 biosynthesis in normal adult tissues; heme may, however, become rate limiting in these processes when heme biosynthesis is chronically compromised during prolonged exposure to environmental agents which selectively inhibit heme biosynthetic capability. This relationship is most clearly demonstrated in livers of rats fed the hepatocarcinogen, ethionine, and it is anticipated that livers of these animals may serve as a useful model in which to study the effects of impaired heme biosynthesis on hemoprotein function resulting from exposure to a variety of environmental hematotoxic agents.

Studies with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) have shown that this substance, although extremely toxic in some organ systems, does not induce

hepatic heme biosynthesis in mammals. This observation suggests that TCDD-related porphyria may be incurred via impairment of porphyrin metabolism or some other process which is not directly related to heme biosynthesis in mammals. In contrast, TCDD does induce the synthesis of a specific form of the cytochrome P-450 (P-448), and recently concluded P-448 reconstitution studies performed in vitro with heme-depleted liver homogenates from $CoCl_2$-treated rats suggest that the mechanism of TCDD induction of P-448 synthesis involves initial induction of apocytochrome P-448, which then combines in vitro with added hemin to form the functional cytochrome. Presumably, TCDD induces the synthesis of cytochrome P-448 in vivo through this mechanism, the apoprotein combining with available heme to form the active cytochrome de novo.

Studies of the effects of trace metals on heme biosynthesis and degradation during prolonged exposure in mammals have revealed methyl mercury, lead and arsenic compounds produce specific alterations of certain enzymes of the heme metabolic processes. These effects are manifested in turn by changes in excreted and circulating levels of heme precursors, which are specific for the metal involved. Results of these studies are being utilized in the design of predictive tests for assessing sub-toxic exposure to specific trace metals in human populations.

Studies concerning the role of heme in the regulation and functional activation of hemoproteins in mammalian tissues will continue. Particular emphasis will be placed on understanding factors affecting the synthesis and regulation of mitochondrial and microsomal cytochromes in mammalian tissues and the effects of chronic exposure to agents which impair heme biosynthetic capability on these processes. Specific studies will be focused on investigation of the mechanisms by which various trace metals selectively interact with heme biosynthetic and metabolic processes, and on further elucidation of the mechanism of TCDD induction of mixed function oxidase activity in mammalian liver. In addition, findings from these studies will be utilized in the design of tests for assessing the potentially toxic effects to humans who are chronically exposed to hematotoxic agents in the environment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the mechanisms by which environmental agents selectively exert toxic effects on cellular growth and function is essential to the development of a biochemical basis for both understanding and preventing chronic organ toxicity. The proposed studies are designed to develop such knowledge in the area of heme biosynthetic function through investigation of the mechanisms of regulation of heme metabolic and degradative processes in mammalian tissues, and through elucidation of the effects of prolonged exposure to hematotoxic agents on the biosynthesis and function of specific regulatory hemoproteins. It is anticipated that these studies will contribute to an enhanced understanding of the problems and mechanisms of chronic organ toxicity resulting from impaired heme biosynthetic capability and hemoprotein function, and will promote the more efficacious identification of potentially harmful hematotoxic

environmental substances. In addition, these studies will enhance the design of clinically useful tests for assessing subtoxic exposures to specific environmental agents in human populations.

PUBLICATIONS

Woods, J.S.: Studies on the Rate-Limiting Role of δ-Aminolevulinic Acid Synthetase in Heme Biosynthesis in Fetal Rat Liver. In Karger, S. (Ed.): Porphyrins in Human Disease. Basel, 1976, pp. 86-97.

Woods, J. S.: Developmental aspects of hepatic heme biosynthetic capability and hematotoxicity. Biochem. Pharmacol. 25: 2147-2152, 1976.

Woods, J. S.: Heme regulation of cytochrome oxidase synthesis in fetal rat liver. Molec. Pharmacol. 13: 50-59, 1977.

Fowler, B. A. and Woods, J. S.: The transplacental toxicity of methyl mercury to fetal rat liver mitochondria: Morphometric and biochemical studies. Lab Invest. 36: 122-130, 1977.

Fowler, B. A. and Woods, J. S.: Ultrastructural and biochemical changes in renal mitochondria following chronic methyl mercury exposure: The relationship to renal function. Exp. Molec. Pathol. (in press).

Woods, J. S. and Fowler, B. A.: Renal porphyrinuria during chronic methyl mercury exposure. J. Lab. Clin. Med. (in press).

Fowler, B. A., Woods, J. S., and Schiller, C.M.: The ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ. Health Perspec. (in press).

Schiller, C.M., Fowler, B.A., and Woods, J.S.: Arsenic effects on pyruvate dehydrogenase activation. Environ. Health Perspec. (in press).

Woods, J. S. and Fowler, B. A.: Effects of chronic arsenic exposure on hematopoietic function in adult mammalian liver. Environ. Health Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70200-04 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Morphologic and Biochemical Effects of Toxic Metals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Biochemical Toxicology Group

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.6	4.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

When animals are chronically exposed to different trace metals, each metal produces a biological response profile which specifically characterizes exposure to that metal. It is the objective of these studies to assess and characterize response profiles based on a thorough understanding of subcellular mechanisms of metal toxicity and specifically to (1) define and correlate ultrastructural and biochemical responses in vivo which characterize exposure to toxic trace elements following chronic exposure and (2) develop early, specific, and sensitive, biochemical testing procedures that may be used to evaluate human populations exposed in utero or as adults to environmentally important toxic elements. Specific metals and areas of interest include the biochemical effects of methyl mercury, cadmium, arsenic, selenium, lead, and lead plus cadmium and arsenic on mitochondrial structure and function. Enzyme activities associated with mitochondrial membranes have been found to show early and pronounced changes in mitochondria of rats and mice following chronic exposure to methyl mercury and arsenic. Further development of these biochemical response profiles should permit development of metal-specific biochemical testing procedures which accurately assess a biological response to these agents prior to overt toxicity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy; x-ray microanalysis; biochemical assays of mitochondrial respiration; and microsomal, cytosol, and mitochondrial enzymes. Sephadex and DEAE chromatography, electrophoresis, and amino acid analysis of metal binding proteins from mammals and marine shellfish. Tissue analysis for metals by atomic absorption spectroscopy and proton induced x-ray emission analysis.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Arsenic. Combined ultrastructural and biochemical studies on liver mitochondria from rats and mice exposed to 0, 20, 40, or 85 ppm arsenate (As^{+5}) for 6 weeks produces *in situ* mitochondrial swelling which was found by morphometric analysis to be most pronounced in hepatocytes near the periphery of the liver lobule. This ultrastructural effect was correlated with decreased state 3 respiration and respiratory control ratios for pyruvate/malate but not succinate-mediated respiration. Further studies on the mechanism of this effect disclosed inhibition of pyruvate dehydrogenase activity and perturbation of the processes which regulate activity of the pyruvate dehydrogenase complex. Studies concerning the effects of prolonged arsenate exposure on the hepatic mitochondrial heme biosynthetic pathway disclosed alteration of several enzymes of this pathway with resultant uroporphyrinuria. These results are distinct from those observed with lead or methyl mercury exposure and suggest a selective effect of arsenic which may have utility in the pre-toxic detection of arsenic damage to exposed human populations.

(2) Methyl Mercury. Rats and mice were given access to drinking water containing 0, 3, 5, or 10 ppm mercury as methyl mercury hydroxide for 6 weeks. Ultrastructural and biochemical studies on livers and kidneys from these animals disclosed that renal mitochondria are more adversely affected than are hepatic mitochondria and that mice show a more pronounced and uniform response to this regimen than do rats. State 3 respiration and respiratory control ratios are decreased in mitochondria of both species. Activities of renal mitochondrial marker enzymes were found to show only moderate measurable changes with the exception of those in the heme biosynthetic pathway, which were markedly altered. This effect was found to result in a porphyrinuria characterized by a greatly elevated excretion of coproporphyrin. It has been proposed that these observations may also have utility in the assessment of mercury poisoning in human populations.

(3) Cadmium. The synthesis, composition, and turnover of cadmium-specfic metal-binding proteins were studied in rats injected for 1 month with 0.2 mg/kg of cadmium chloride and in oysters exposed to cadmium in sea water at a concentration of 0.1 mg/l for 2 weeks. The cadmium-binding proteins isolated from both species were found to be of similar molecular weight but with different spectral and electrophoretic properties and amino acid compositions.

(4) Interactions between Lead, Cadmium and Arsenic. An interaction study was conducted on rats exposed to dietary combinations of lead (200 ppm), cadmium

(50 ppm), and arsenic (50 ppm) as either arsenilic acid or sodium arsenate for 10 weeks. A variety of clinical chemistry and ultrastructural parameters were assessed. The most pronounced interactive effect involved urinary porphyrin excretion patterns which indicated that some lead-cadmium and lead-arsenic interactions do occur with respect to mitochondrial toxicity and to changes in porphyrin excretion patterns. Lead produced significant increases in excretion of urinary ALA and coproporphyrin but not uroporphyrin. Simultaneous administration of cadmium with lead caused a decrease in urinary ALA excretion but not that of coporporphyrin. Cadmium alone did not markedly alter urinary excretion of any of the measured porphyrins. Arsenic as either the organic or inorganic form produced no change in ALA excretion but caused marked increases in urinary uroporphyrin and to a lesser degree coproporphyrin thus confirming earlier findings with this element. Lead in combination with arsenic produced an additive effect on coproporphyrin excretion but no alteration of the arsenic effect on uroporphyrin excretion indicating that the arsenical effect an uroporphyrin excretion is rather specific.

Further studies on the subcellular mechanisms of trace metal toxicity are planned for arsenic, selenium and lead. A contract for the screening of selected biochemical and physiological parameters following chronic oral or inhalation exposure to these elements is to be initiated. Based on the results of these studies, tests of potential clinical utility will be devised to detect subtoxic exposure of human populations to selected trace metals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal toxicity following chronic exposure by both ultrastructural and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations.

PUBLICATIONS

Fowler, B. A.: Environmental arsenic toxicology. In Carnow, B. W. (ed.): Health Effects of Occupational Lead and Arsenic Exposure - A Symposium. U.S. Dept. of Health, Education, and Welfare, Public Health Service Pub. No. 76-134, Washington, D. C., Government Printing Office, February, 1976, pp. 248-252.

Fowler, B. A.: Lead levels in dover sole collected in southern California coastal waters. In Carnow, B. W. (ed.): Health Effects of Occupational Lead and Arsenic Exposure - A Symposium. U. S. Dept. Of Health, Education, and Welfare, Public Health Service Pub. No. 76-134, Washington, D. C., Government Printing Office, February, 1976, pp. 141-145.

Fowler, B. A.: The Toxicology of Environmental Arsenic. In Mehlman, M. and Goyer, R. A. (eds.): Advances in Modern Toxicology III. The Trace Metals. Horizon Publishing Co., 1977, pp 79-122.

Fowler, B. A. and Woods, J. S.: The transplacental toxicity of methyl mercury to fetal rat liver mitochondria: Morphometric and biochemical studies. Lab. Invest. 36: 122-130, February, 1977.

Faeder, E., King, L., Chaney, S., Bruce, R., Hinners, T., and Fowler, B. A.: Biochemical and ultrastructural changes in liver in cadmium-treated rats. Toxicol. Appl. Pharmacol. 39: 473-487, March, 1977.

Woods, J. S. and Fowler, B. A.: Renal porphyrinuria during chronic methyl mercury exposure. J. Lab. Clin. Med. (in press).

Fowler, B. A., Woods, J. S., and Schiller, C. M.: The ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ. Health Perspec. (in press).

Schiller, C. M., Fowler, B. A., and Woods, J. S.: Arsenic effects on pyruvate dehydrogenase activation. Environ. Health Perspec. (in press).

Woods, J. S. and Fowler, B. A.: Effects of chronic arsenic exposure on hematopoietic function in adult mammalian liver, Environ. Health Perspec. (in press).

Mahaffey, K. R. and Fowler, B. A.: Effects of concurrent administration of dietary lead, cadmium, and arsenic in the rat. Environ. Health Perspec. (in press).

Fowler, B. A.: International conference on environmental arsenic: An overview. Environ. Health Perspec. (in press).

Fowler, B. A. and Woods, J. S.: Ultrastructural and biochemical changes in renal mitochondria during chronic oral methyl mercury exposure: The relationship to renal function. Exp. Molec. Pathol. (in press).

Fowler, B. A.: The general subcellular effects of lead, mercury, cadmium, and arsenic. Environ. Health Perspec. (in press).

Engel, D. W. and Fowler, B. A.: Accumulation and biological effects of copper and cadmium on marine shellfish. Proceedings of the technical review of the interagency Marine Research Program associated with energy development. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70220-04 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Developmental Exposure to Lead

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. Kimmel	Senior Staff Fellow	LET NIEHS
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Environmental Biology and Chemistry Branch, NIEHS
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LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Behavioral Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The developmental effects of lead are being studied using an interdisciplinary approach to evaluate and compare various toxicological parameters with an emphasis on neurobehavioral measurements. A broad battery of observations focussed on physical and reflex development, and behavioral functions, is being employed to determine the neurotoxic potential of lead. These observations are being related to teratogenicity, body burden of lead, histopathologic and ultrastructural changes, effects on the biochemistry of target tissues (brain and kidney), as well as functional changes in other systems. This multidisciplinary approach will be evaluated in terms of its usefulness for routine assessment of compounds with unknown neurotoxic potential.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats obtained at weaning are being exposed to the following doses of lead (as lead acetate) in deionized drinking water: 0, 0.5, 5, 25, 50, and 250 ppm. Exposure is continued for 6-7 weeks at which time animals are mated; then exposure continues through pregnancy, lactation, and subsequently to the offspring following weaning. At 21 days of gestation some animals are sacrificed and a routine teratology exam is performed. Other animals are sacrificed at various times throughout the exposure period and blood, brain and bone collected for analysis of lead by atomic absorption spectroscopy. Another group of animals is allowed to litter, and offspring are examined for physical milestones (e.g., eye opening, pinna detachment), reflex ontogeny (e.g., righting, visual placing), and later for locomotor coordination, activity, learning acquisition of several responses, and sexual behavior. Additional evaluations include (1) measurement of brain acetyl-and butyrylcholinesterase in neonates and weanlings, (2) evaluation of reproductive function in mated offspring, and (3) evaluation of immunologic competence in the young (Project No. Z01 ES 30007-05 EBCB). Offspring are exposed until 9 months of age at which time they are sacrificed and blood samples are taken for lead analysis, routine hematologic examination and clinical chemistries. Body and organ weights are obtained; tissues are taken for histopathology and electron microscopy; and kidneys are homogenized and used to study heme biosynthesis and mitochondrial respiratory activity.

MAJOR FINDINGS AND PROPOSED COURSE: Lead doses of 50 and 250 ppm produce overt toxic effects on body weight, physical and behavioral development and biochemical and morphologic parameters. At 25 ppm, there is an effect on time of vaginal opening in parent and F_1 females and in the immunologic competence. In 9-month-old male rats, there is a significant effect on kidney weight at all doses and on changes in renal morphology starting at the 5 ppm dose level. These changes in the kidney are the most subtle effects due to lead observed so far. Further observations on behavior are presently underway and several of the evaluations noted here will be replicated in subsequent groups of animals. The evaluation of the results of this study will be used to determine appropriate methods for screening of environmental agents for neurobehavioral toxicity. In addition, areas will be identified which require development of new and better methods for assessing behavior. This project will be terminated by the end of FY 1977.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is an immediate need for the study and evaluation of toxicant effects on behavior and the assessment of such effects as early indicators of toxicity. These studies relate directly to the tremendous problems of congenital mental retardation and abnormal behavior in children for which there is, in most cases, no known etiology. In terms of relevance to the field of toxicology, many questions are being addressed in this project which relate to sensitivity, approach and validity of presently used toxicological evaluations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70280-04 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Plasma Membranal Enzymes and Regulation of Mammalian Embryo Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	E. Soares	Senior Staff Fellow	LEM NIEHS

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.1	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this investigation is to attempt to ascertain the nature of developmental regulatory systems whose modification during embryogenesis may result in altered neural tube development (e.g., anencephaly, exencephaly, or spina bifida). The studies focus on: (1) the problems associated with staging teratogenic responsiveness in offspring of the rat or mouse; (2) characterization of the behavior of those plasma membranal enzymes (e.g., prostaglandin synthetase, (Na^+-K^+)ATPase, and adenyl cyclase) which are likely to play a significant role in determining responsiveness of embryonic cells to teratogenic stresses, namely, those due to deficiencies in essential nutrients, to drugs and to environmental agents.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Feeding studies require the use of defined diets and occasional diet modification (addition of antimetabolites, drugs, etc.). Also required are facilities for mating and for anatomical (teratology) examination. Behavioral studies of embryo-fetal, uterine, and placental prostaglandin synthetases are performed using analytical techniques which can permit chemical identification of the prostaglandins.

MAJOR FINDINGS AND PROPOSED COURSE: Endogenous levels of prostaglandins E_2 and $F_{2\alpha}$ (determined by radioimmunoassy) in rat decidua-conceptus complex (DCC) tissue increased progressively on pregnancy days 9, 10 and 11, then started to level off on day 12.

Prostaglandin (PG) synthesis ability of decidua, which accounted for essentially all of the production of PGs in the DCC, increased rapidly on pregnancy days 9 through 11, leveled off on day 12, then declined rapidly on day 13. Since the PG synthetic capability of each DCC on pregnancy days 11 and 12 was about 3% to 6% of that for the entire uterus on day 21, it was assumed that such synthetic capability might be of physiological significance (since the average litter size was 10 to 12, the PG synthesis ability of day 11 or 12 DCCs on these days amounts to 30% to 80% of that of the day 21 uterus).

In order to determine whether modification of decidual PG synthetase activity could be causally-related to teratogenicity of aspirin or salicylate, the PG synthetase ability of the DCCs and endogenous PGE_2 and $F_{2\alpha}$ levels were determined on pregnancy day 11 subsequent to in vivo administration of indomethacin (at a dosage which was neither teratogenic nor apparently toxic) and of nonteratogenic and teratogenic doses of aspirin on pregnancy day 10. Results of this study indicated that there was no causal relationship between teratogenicity of aspirin and modification of measured PG levels or PG synthetase.

Several efforts were undertaken to try to confirm the hypothesis that abnormal fetal development resulting from maternal dietary deficiency in pantothenate is genetically determined. Unfortunately, by the time the definitive experiment was to be conducted, a large percentage of the rats (used in the preliminary diagnostic breeding effort) had acquired bacterial (PPL) infection; furthermore, the particular batch of commercially prepared diet had not been suitably pre-tested. As a result, this crucial experiment resulted in a high incidence of litters in which all embryos were resorbed, a result which had not previously been obtained in those same control animals when they were tested several months earlier. This project was discontinued as of September 30, 1976.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Alterations in rat embryonic neural tube development which results from a mild teratogenic perturbation such as maternal consumption of a pantothenate deficient (PD) diet or of a PD diet supplemented with aspirin only during

pregnancy appeared to be genetically determined. Only a better understanding of the biochemical basis(es) for abnormal embryonic development might permit anticipation of and/or protection against agents responsible for such development.

Our efforts have provided an indication of the variability of prostaglandin synthesis by uterine endometrial and decidual tissue. By analogy to more highly differentiated cell types, prostaglandin synthetase, adenyl cyclase, and other plasma membranal enzymes may be anticipated to play important roles in regulation of embryonic differentiation; however, decidual prostaglandin production does not appear to represent a phenomenon which is directly involved in alteration of embryo development in the rat.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70300-07 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. Moore	Chief	MAPB NIH
	P. Mortillaro	Cinematographer	MAPB NIH
	J. Romine	Cinematographer	MAPB NIH
	A. Godwin	Photographer	MAPB NIH
	G. Alderson	Oral Pathologist	AFIP
	C. J. Davis	Renal Pathologist	AFIP
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Med. Arts & Photo. Branch, NIH — Pulmonary Branches, AFIP

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project seeks to develop the opossum Didelphis virginiana as a biomedical model by (1) characterizing the normal neonatal and developing opossum anatomically and physiologically and (2) determining the pathophysiologic response of these animals to selected enivronmental toxins and carcinogens.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Special pharmacological-histologic techniques, light and electron microscopy, and standard biochemical methods of ultracentrifugation, thin-layer chromatography, and gas chromatography are utilized.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Breeding and Laboartory Management of the Opossum. Paper No. 4 in the series on the opossum titled: "Some Aspects of the Reproductive Behavior of the Opossum (Didelphis virginiana) in Captivity" has been submitted for publication.

(2) Opossum Film. Completion of the opossum films (entitled: "The American Opossum as a Biomedical Model." Part I: "The Neonatal and Developing Opossum" and Part II: "The Adult Opossum") continues to be delayed due to unavailability of resources. NIEHS management previously indicated that funds would not be released for the purpose of completing this work until the carcinogenesis work was complete. A request for funds is in preparation.

(3) Biologic Characterization of the Opossum. (a) Studies to isolate and characterize growth hormone and prolactin from the opossum pituitary in collaboration with Dr. U. J. Lewis, Scripps Institute, LaJolla, California, are completed. These hormones were identified and characterized electrophoretically. The data are being prepared for publication. (b) All other studies have been aborted due to withdrawal of NIEHS support.

(4) Carcinogenesis Study. (a) Work continues on the characterization of the lesions induced in developing opossums with ethylnitrosourea (100 mg/kg).

Data was compiled on the incidence and distribution of non-embryonal tumors in comparison to embryonal tumors induced in the opossum with ENU.

A spectrum of mesenchymal and epithelial neoplasms comprising 43 different tumors distributed among 21 different sites was found in the ENU treated opossum neoplasms. Seventy-three percent of the tumor bearing animals had more than one primary neoplasm. Neoplasms (small nephroblastomas) were found in two control animals.

Seven types of embryonal tumors were induced in six different sites. These tumors were closely analogous in morphology and biology to their counterparts in the human. With the exception of the ameloblastoma, one of two odontogenic tumors, a correlation was apparent to a varying degree between susceptibility to the embryonal tumors and the state of morphologic maturation of the presumed target tissues. Three of these neoplasms, an intraocular neoplasm (teratoid medulloepithelioma, a tumor of the neuron (ganglioglioma), and a tumor of the jaw (odontogenic myxoma) are of interest because they have not to our knowledge been previously induced experimentally. These neoplasms as well as the embry-

onal tumor of the kidney (nephroblastoma) and a second type of embryonal jaw tumor (ameloblastoma) were notable for their morphologic similarity to analagous human cancers.

These lesions were frequently present in tumor bearing animals. A single teratoma of the neck was found in an animal given ENU at birth. Two types of hamartomas were found. Hemangiomas were present in 11 different sites and osteomas were seen in 3 different skeletal locations. Malformations in the form of cysts of the kidney and of hypoplasia of the genitalia were also found in high incidence in the ENU treated opossums.

In the kidney, an embryonal neoplasm was occasionally present at the same site as a malformation. Thus, in two animals given ENU every other day from day 7 to 13, neoplastic and teratologic changes were found in the same kidney. Such a coincidence of tumor and malformation has not been previously reported under experimental conditions despite its occasional occurrence in the human.

Thirty-six different tumors (12 benign and 24 malignant types) analagous in varying degrees to tumors which normally appear during adult life in man were induced in 20 different sites. The incidence of most of these tumors was too low to determine whether there was a correlation between susceptibility and age of the host at time of tumors induction. However, a number of the neoplasms with high incidence such as the benign tumors of the thyroid, liver, stomach, and the malignant tumors of the jaw, liver, and lung were induced throughout the 16 week period of exposure. Several of the "adult type" tumors were of particular interest because of their malignancy. Thus, the adenocarcinoma of the lung, the hepatocellular carcinoma of the liver, the cholangiocarcinoma of the bile ducts the melanoma of the skin, and the adenocarcinoma of the large and small intestines metastasized to various sites. The pulmonary adenocarcinoma, and the melanoma of the skin showed the most widespread metastases. A granulocytic leukemia was also highly malignant with massive infiltrates of tumor cells in liver, lymph nodes, kidney, and lung.

"Adult type" tumors of special interest because of their rarity in eutherian laboratory species or because their target sites appear resistant to neoplastic transformation in these species, were the basal cell carcinoma of the skin (1 case), the cystadenocarcinoma of the liver (4 cases), the ductal adenoma of the pancreas (3 cases), the adenocarcinoma of the prostate (2 cases) and the cutaneous papilloma (35 cases).

Chemical induction in the opossum, at stages of development comparable to in utero stages in man and other eutherian species, of a broad spectrum of tumors with a wide range of latencies and malignancies, supports the emerging concept that in utero exposure to carcinogens in humans may account for a greater proportion of tumors than previously believed. This concept is fueled by experimental evidence that transplacental exposure to a carcinogen tends to enhance the effect of subsequent exposures during postnatal life to the same or some other carcinogen and by the recent discovery of "adult type" neoplasms (clear cell adenocarcinomas) in the vaginas of adolescent girls born to mothers given diethylstilbestrol during pregnancy.

These data were presented at the Third International Conference on Detection and Prevention of Cancer in April 1976.

Correlations between susceptibility to embryonal tumors and differentiation of the target tissue were described at the Conference on Perinatal Carcinogenesis in January 1976. (Please see Annual Report for FY75.)

(b) Collaborative Studies and Consultation. The principal investigator has been asked to collaborate in several carcinogenesis studies utilizing the opossum, being conducted or planned at other institutions.

(1) In collaboration with Dr. Bernd Hamprecht of the Max Planck Institute of Biochemistry in Munich, West Germany, a small opossum breeding colony has been successfully established with the objective of reproducing the brain (ganglioglioma) and eye (teratoid medulloepithelioma) neoplasms induced with ethyl nitrosourea. (Please see Annual Reports for FY74 and 75.) Tissue from these two tumor types will be (a) frozen as part of a tumor tissue bank, (b) innoculated into nude mice and (c) placed in tissue culture for morphological, biochemical and electrophysiological studies of neoplastic neurons in vitro.

(2) The principal investigator functions as a consultant to the Laboratory of Gastrointestinal Physiopathology, Department of Medicine, University of Lenven, (Drs. J. Jaspens and G. Vantrappen), Belgium in a study of the physiology of peristaltic contraction in the esophagous of the opossum.

(3) The principal investigator is a consultant to the Netherlands Central Institute for Brain Research Amsterdam, Netherland in a comparative morphologic study of the opossum pineal gland (Dr. P. Pevet) and work on nerve regeneration (Dr. R. Baber).

(4) The principal investigator has been asked to collaborate in establishing a research program to reproduce embryonal tumors of the jaw using the opossum neonate by the Department of Pathology, University of Alabama Medical School, Birmingham, Alabama (Dr. S. Hoffman).

(5) The principal investigator has been asked to collaborate in establishing a research project involving the induction of retinoblastic neoplasms in the neonatal opossum at the Eye Institute of Retina Foundation, Harvard Medical School (Dr. Mukai).

c. Contract Studies. Attempts to re-establish the opossum carcinogenesis study with NCI support continues. Late in 1975 the Division of Cancer Control and Prevention of the NCI offered to transfer funds to NIEHS in order to re-establish the opossum carcinogenesis study. The NIEHS refused to accept funds directly but agreed to allow the principal investigator to function as a co project officer on a formal contract. Proposals submitted to DCCP, NCI in September 1976 received a positive review for need, relevance, and priority for contract effort. The nature of the contract procedure has resulted in repeated delays. Attempts are underway to identify a contractor.

The proposed course of this work is as follows:

(1) Carcinogenesis Study. Histologic preparation of tissues and histopathologic interpretation and classification of lesions will continue as resources allow.

(2) Opossum Films. Completion of the films will depend on the availability of pregnant opossums and technical and fiscal support.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The opossum model provides an opportunity to directly evaluate the relationship between susceptibility to embryonal carcinogenesis and differentiation of the target tissue. The model may also be of value in exploring the apparent interrelationship among oncogenesis, teratogenesis, and mutagenesis in the absence of the major handicap intrinsic to the eutherian animals, namely, the impossibility of distinguishing direct carcinogen induced teratologic mutagenic changes from identical lesions which are the indirect result of adverse physical and physiologic effects of the carcinogen on the maternal and fetoplacental unit. In the semiembryonic, semifetal opossum orally or parenterally exposed to a carcinogen, mutations and malformations can only be either carcinogen induced or spontaneous.

PUBLICATIONS

Jurgelski, W., Hudson, P.M. and Falk, H. L.: Tissue differentiation and susceptibility to embryonal tumor induction by ethylnitrosourea in the opposum. Proc of the Conf. on Perinatal Carcinogenesis (Rice, J.M. ed.) U.S. Govt. Printing Office (in press).

Jurgelski, W., Hudson, P. M. and Falk, H. L.: A new animal model for the direct induction of neoplasms during embryonic and fetal development. Proc. of the 3rd Int. Symp. on Detection and Prevention of Center (Nieburgs, H.E., ed.) (in press).

Jurgelski, W., Hudson, P. M. Falk, H. L., Zimmerman, L. E., Henry, J. M. and Palmer, N.: Experiemntally induced pediatric tumors in the opossum - a clinicopathological and histopathological comparison with analogous neoplasms of man and other species. Tumors of Early Life in Man and Animals. (Severi, L. ed.) (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70800-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neurochemical Correlates of Behavioral Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. E. Wilson	Research Chemist	LET NIEHS
OTHER:	E. A. Zeller	Visiting Scientist	LET NIEHS
	A. Agrawal	Visiting Fellow	LET NIEHS
	C. L. Mitchell	Pharmacologist	LET NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Behavioral Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

In order to obtain a better understanding of the neurochemical bases for behavior modification resulting from environmental stresses, we have undertaken studies concerned with (a) alterations in levels and characteristics of enzymes which, because they are rate limiting, may provide critical regulation of neurotransmitter levels (e.g. monoamine oxidase, tyrosine hydroxylase, etc.) and (b) the extents to which neurotransmitter concentrations may be modulated or altered in those parts of the central nervous system which are thought to be involved in behavior regulation. In the studies involving monoamine oxidase (MAO), we are investigating the behavioral consequences of inhibiting MAO types A and B as well as several of the characteristics of MAO types A and/or B responsiveness to various substrates and inhibitors.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Behavioral testing (i.e. locomotor activity in an open field) is performed prior to, then after, administration of type-specific monoamine oxidase (MAO) inhibitors to rats. Subsequently, levels of types A and B of MAO are determined in brain, heart, liver and kidney from the animals. MAO types A and B are determined in tissues by using a radioisotope technique. Additional techniques -- namely, UV-spectrometry and fluorescence spectrometry -- are employed to confirm the radioisotope technique and to establish kinetic characteristics of MAO in the presence of various substrates and inhibitors.

MAJOR FINDINGS AND PROPOSED COURSE: Our in vitro studies have permitted the following: (a) development of the first real separate quantitative determination of MAO types A and B in a single tissue analysis; (b) recognition of a technique which may permit activation of MAO activity; (c) accumulation of kinetic data which supports the hypothesis that the main difference between MAO types A and B concerns the geometry of the enzyme's binding sites.

In the in vivo studies, two acetylenic monoamine oxidase (MAO) inhibitors, deprenyl--a fairly specific inhibitor of MAO type B--and clorgyhine--a fairly specific inhibitor of MAO type A--have been administered to several groups of male rats over 7 to 12 day intervals at dosages which led to 75% to 95% inactivation of the responsive type of whole brain MAO. Locomotor activity in an open field test of animals treated with deprenyl and/or clorgyhine did not appear to differ appreciable from that observed among controls. In addition to inactivation of brain MAO activities, these acetylenic compounds inhibited the MAO activity of heart (99+% MAO type A) and of liver (40% to 60% MAO type A) by 50% to 90%. It is not presently known whether modification by deprenyl or clorgyline of MAO in tissues other than brain might influence locomotor activity.

At present, efforts are underway to determine whether dimunition in brain MAO activity might lead to a transitory modification of locomotor activity for which compensatory alternate metabolic pathways might emerge and come into prominence.

With suitable technical assistance it would be desirable to inject inhibitor solutions directly into the brain ventricles in order to minimize inactivation of MAO activity in tissues outside of the CNS, thus, avoiding the possibility of observing altered behavioral response to the acetylenic MAO inhibitors. It would also be desirable to be able to subject the animals to several additional behavioral tests when facilities become available.

Future efforts will involve combining drug treatment with administration of amino acids (e.g. DOPA, tryptophan methionine, etc) in a manner similar to that previously used in studies involving iproniazid, phenylcyclopropylamine and several other MAO inhibitors.

In order to permit neurochemical measurements to become more meaningful in a behavioral context it will be necessary to determine what happens in various small localities within the CNS. This will necessitate development of techniques for recovering small parts of the brain and for measurement of appropriate biochemical parameters. In this regard, although techniques are presently available for quantitative analysis of femtamolar (10^{-15}M) quantities of dopamine and noradrenline, appropriate methodology has not been developed for determination of endogenous levels of other putative transmitters, of neurotransmitter metabolizing enzymes, of transport systems, of receptors, etc.

In those instances where behavior modification might be crucially decided by a particular neuronal cell type or at a crucial locus in the CNS, nerve cell culture should be developed in order to provide a more convenient test system for testing of potential environmental agents as well as for obtaining further insight into other biochemical properties of such cultures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Neurotoxic and behavioral effects may be associated with the alteration of cerebral biogenic amines, or their metabolism, or the disposition of their metabolites. This conclusion is based (1) on the wide occurrence of biogenic amines in the CNS, (2) on the transmitter function of several amines, and (3) on the clinical and pharmacological experience that modulations of the aminergic systems may lead to serious changes in the affective state and to neurologic and psychiatric disorders. The investigation of aminergic systems, therefore, appears to be a promising approach to the elucidation of some phenomena of behavioral toxicology. However, in view of the complexity of these systems the task of unraveling their contributions to behavioral changes is an enormous one. It can be simplified somewhat by directing the effort toward rate-limiting steps such as hydroxylation of tyrosine and tryptophan or oxidative deamination by monoamine oxidase. As a first approach we chose to study the latter.

Levels of monoamine oxidase are known to vary with age and sex in various tissues from humans and experimental animals. MAO variations have also been found to reflect responsiveness of regulatory systems in various tissues to physiological steroids including the sex hormones. In order to ascertain whether, and the extent to which, modification of tissue levels of monoamine oxidase might reflect a significant behavior regulating phenomenon, an effort was undertaken to determine whether it might be possible to simulate behavioral characteristics which have previously been hypothesized to reflect biogenic amine metabolic capabilities in old vs. young animals or in male vs. female animals. In view of the importance of regulation of serotonin and/or catecholamine neurotransmitter levels in connection with human behavior, it is obviously desirable to attempt to find a suitable animal model.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70805-01 LET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neurotoxicological Effects of Hexabrominated Biphenyls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Pharmacologist	LET, NIEHS
OTHER:	P. Cabe	Senior Staff Fellow	LET, NIEHS
	D. Mitchell	Staff Fellow	LET, NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch, NIEHS

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Behavioral Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ⊦

The purpose of this project is to determine, as quantitatively as possible, the effects of oral, subacute doses of firemaster (a mixture of polybrominated biphenyls, PBBs) and 2,2',4,4',5,5'-hexabromobiphenyl (HBB) in a battery of simple, diagnostic tests designed to detect changes in various functions of the central nervous system (CNS).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Male and female albino rats are being given daily, oral doses of 0.03, 0.3, 3, or 30 mg/kg of firemaster, HBB, or vehicle for at least 60 days. The rats and those mice receiving 30 mg/kg/day of either compound or vehicle will be evaluated in a battery of tests on days 28 and 29 of dosing and then retested 30 days later. The test battery will measure drug-related changes in autonomic-parasympathetic nerve function, sensorimotor function and CNS excitability and emotionality.

MAJOR FINDINGS AND PROPOSED COURSE: The study is currently in progress and no data are available. If the battery is useful in its current form it can be employed immediately to study other compounds. However, some portions of the battery may need to be revised and validated prior to use with new agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results from this project will be used in the development of appropriate methods to screen for neurotoxic effects following chronic low-level exposures to environmental agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70810-01 LET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Etiology of Pica in the Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. Mitchell Staff Fellow LET, NIEHS

COOPERATING UNITS (if any)

Zoology Department, Duke University

LAB/BRANCH

Laboratory of Environmental Toxicology

SECTION

Behavioral Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This series of experiments seeks to determine the necessary and sufficient factors in the etiology of pica (the consumption of seemingly non-nutritive substances) and to validate the utility of pica as a behavioral assay of toxicosis. A variety of toxic substances have been shown to cause laboratory rats to engage in geophagia (earth eating). Moreover, rats poisoned with logarithmic doses of toxic substances consume amounts of non-nutritive substances proportionate to the amount of poison administered. Other factors demonstrated to be involved in the etiology of pica include gastrointestinal malaise and the acquisition of a conditioned illness. The objectives of this research are (1) to investigate the etiology of pica, (2) to evaluate pica as a behavioral assay of toxicosis in the rat, and (3) to assess the functional utility of pica during poison-induced illness.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats are maintained with food, water, and a non-nutritive mixture of pharmaceutical grade kaolin (hydrated aluminum silicate) and 1% acacia (Gum Arabic) ad libitum. After a period of adaptation to this regimen, the animals are subject to a variety of illness-inducing treatments including motion sickness, and the administration of toxic substances such as cyclophosphamide, lithium chloride, and methyl mercury. In some cases the treatments are restricted to single acute exposures; in others repeated chronic exposures are employed. Following treatment the subsequent changes, if any, in food, water and kaolin consumption are recorded. Variations in this basic procedure include monitoring the temporal parameters of the various consummatory behaviors, adulteration of the kaolin with various concentrations of lead acetate, and the use of conditioning procedures during treatment.

MAJOR FINDINGS AND PROPOSED COURSE: In a continuing series of experiments it has been determined that simple gastrointestinal malaise in the absence of a deficiency state or acute toxemia will elicit pica, that different strains of both laboratory and wild rats show behavioral variation in their propensity to engage in pica, and that a variety of toxic substances (methyl mercury, cyclophosphamide, alcohol, lithium chloride, and various cardiac glycosides) administered either acutely or chronically will reliably cause laboratory rats to engage in a specific form of pica--geophagia or earth-eating. It has also been determined that in some cases the acquisition of a conditioned illness can be a mechanism whereby the habit of pica is maintained in the absence of a demonstrable physiological cause.

It has also been found that pica can be used as an easily quantifiable behavioral assay of noxious drug effects. In two separate experiments, rats poisoned with logarithmic doses of either Red Squill (a cardiac glycoside) or cyclophosphamide consumed amounts of non-nutritive substances proportionate to the amount of poison administered. In an additional series of experiments, changes in two different consummatory behaviors following lithium chloride poisoning were compared. Decreased food consumption (hypophagia) and increased clay consumption (geophagia) occurred in rats given a single intraperitoneal injection of 0.45 M lithium chloride (381.6 mg/kg). Moreover, clay consumption had no effect on food consumption, indicating that these responses to poisoning are complimentary but independent behaviors. Further differences in hypophagia and geophagia were revealed when rats were given ten repeated intraperitoneal injections of .15 M lithium chloride (127.2 mg/kg/trial) spaced five days apart. Whereas the characteristic deficit in food consumption was remarkably stable across the ten trials (averaging approximately 6 g/rat/trial), clay consumption increased from a low of 1.5 g on the first trial to a high of 15.3 g on the fifth trial. After the fifth trial, geophagia showed the same stability as hypophagia across the subsequent trials.

Further studies on the etiology of pica are planned. For the immediate future, the emphasis will be on the development of pica as a biological assay

of toxicosis. As this work progresses, it is expected that the sensitivity of pica and other consummatory behaviors can be enhanced by modifying the present paradigm to include temporal parameters. A series of experiments designed to assess the possible utility of pica in veterinary medicine is also planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential significance of this research lies in three major areas: As a behavioral assay of toxicosis, as a laboratory model of pica, and as a strategy in rodent control. Knowledge of the rat's naturally occurring illness-response behaviors such as pica could suggest new techniques and enhance existent species-relevant behavioral assays of illness in the rat. The development of a laboratory model will not only clarify various aspects of the etiology of pica but hopefully reveal methods of mitigating its harmful effects, such as the lead poisoning which is often associated with pica among human infants. Finally, a knowledge of the naturally occurring illness related behaviors of the rat should enable one to devise control methods that circumvent the effectiveness of these behaviors in protecting the animal from poisoning and enhance the effectiveness of control strategies.

THE DOW CHEMICAL COMPANY - Midland, Michigan 48640
(NIH-NIEHS-73-2102)

TITLE: Effect of Environmental Components on Reproduction and Ontogenesis

CONTRACTOR'S PROJECT DIRECTOR: B. A. Schwetz, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D., Research Physiologist
Teratology Group, LET

DATE CONTRACT INITIATED: February 5, 1973

CURRENT ANNUAL LEVEL: $357,000

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential in animals of environmental components when administered singly or in combination; (2) to determine species differences in response to teratogens and to study the etiology of such differences; and (3) to investigate the mechanism of action of teratogens and to improve experimental testing and extrapolation to man.

METHODS EMPLOYED: In general, the screening approach outlined by the World Health Organization (Technical Report No. 364, 1967) is being followed with certain modifications to alleviate some of the problems encountered by private industry during the testing of potential therapeutic agents.

MAJOR FINDINGS AND PROPOSED COURSE: Carbaryl, a broad spectrum insecticide, was not teratogenic when given to mice by gavage, or by inclusion in the diet, at doses up to maternally-toxic levels. The response also was negative when piperonyl butoxide or phenobarbital were combined with the carbaryl. However, administration of carbaryl by gavage to the rabbit at the maximum tolerated dose of 200 mg/kg/day resulted in a significant increase in fetal resorptions and omphalocoeles. The latter represent two positive teratogenenic responses.

Combined administration of carbon monoxide (inhaled) and ethanol (imbibed) was not teratogenic in mice or rabbits even at the maximum tolerated dose levels.

Sulfur dioxide was not teratogenic when inhaled alone by pregnant rabbits at 70 ppm for 7 hr/day or when inhaled in combination with carbon monoxide. Mice are more sensitive to sulfur dioxide but a teratogenic potential was not detected in this species either.

Decreased fetal weight and an increased incidence of cleft palates were seen among the offspring of mice that inhaled chloroform between days 8 and 15 of gestation, but not among those exposed between days 1 and 7 or 6 and 15 of gestation. Exposure before day 8 of gestation often resulted in termination of pregnancy.

To publish the results of studies completed; to complete studies currently being conducted on sulfur dioxide, sulfuric acid aerosol, and tetrachloroace-

tone; to determine whether an additional study on chloroform in mice is indicated; and to begin study of hexachlorocyclopentadiene.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most environmental agents have not been tested for teratogenic potential. These studies were undertaken to provide such information on a variety of agents to which man is exposed. Since it is known from studies in laboratory animals that combined administration of two teratogens can potentiate teratogenic response, combinations of non-teratogenic agents were tested to determine whether conditions of increased sensitivity to the developing embryo might be revealed. This information will serve in the formulation of a rational basis for evaluation of the potential hazards to human development produced by the use and exposure of these chemicals.

PUBLICATIONS

Schwetz, B. A., Nitschke, K. D. and Staples, R. E.: Cleft Palates in CF_1-mice After Deprivation of Water During Pregnancy. Toxicol. Appl. Pharmacol. (in press).

ENDOCRINE LABS OF MADISON - Madison, Wisconsin 53707
(NIEHS NO1-ES-6-2111)

TITLE: Effects of Environmental Agents on Male and Female Reproductive Tract Function

CONTRACTOR'S PROJECT DIRECTOR: James Davis, Ph.D.

PROJECT OFFICERS (NIEHS): G. W. Lucier, Ph.D., Head, Develop. Toxicol. Group
Developmental Toxicology Group, LET
J. A. McLachlan, Ph.D., Developmental Toxicologist
Transplacental Toxicity-Reproduction Group, LET

DATE CONTRACT INITIATED: March 20, 1976

CURRENT ANNUAL LEVEL: $206,000

PROJECT DESCRIPTION

OBJECTIVES: A series of experiments are described which will provide information on the effects of 24 selected xenobiotics (6 per annum) on the fertility of mice (male and female) following prenatal or postnatal exposure, and, determine if levels of reproductive hormones have deviated from the norm or if a pathologic condition exists in mice whose reproductive capacity has been altered. Information will be obtained on the possible developmental toxicity of these chemicals, focusing on transplacental carcinogenesis.

METHODS EMPLOYED: Time - Pregnant mice (CD-1) are treated subcutaneously or orally with test compounds at 4 dose levels --each dose level consisting of 15 animals or a total of 75 females per compound. All P_1 females will be allowed to deliver at term and raise their young to weaning. At 8 weeks of age, representative F_1 female offspring will be placed on a Forced Breeding Study for the remainder of their reproductive life to determine their fertility. Litters (F_2 generation) produced in the Forced Breeding STudies will be examined for gross abnormalities and weaned. Representative F_1 females will be sacrificed after 3 litters and at the termination of the experiment for examination of gross and microscopic pathology and for determination of circulating levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), estrogens and progestins.

In females the contractor will primarily evaluate the effect of each compound on: the length of the estrous cycle, number of matings and litters per reproductive lifetime, number of young per litter, number of abnormal young produced, average weight of young. Secondary information which will be collected and evaluated will be levels of reproductive hormone and pathologic condition and neoplastic changes in affected animals.

Selected F_1 males from each P_1 female will be mated to 20 females (2 females every 5 days) in two mating studies. Prior to the first 25 day mating series and following the completion of the second, selected F_1 males will be sacrificed.

Pathology studies are done on the reproductive organs, and levels of reproductive hormones (LH, FSH, androgens and testosterone) are measured in selected animals. Nine days after removal from the male, one female of each pair is autopsied and the number of implants and early and late resorption noted. The second female is allowed to deliver at term, the F_1 young counted, examined and weighed.

In males the contractor primarily evaluates the effect of prenatal treatment effect of each compound on: number of successful matings, number of successful litters sired, number of young per litter sired, number of abnormal, young sired. Secondary information collected and evaluated will be levels of reproductive hormones and pathologic conditions in affected male animals.

In the second study outlined, male mice (9-10 weeks old, Cd-1) are treated with a compound at 3 dose levels (10 animals per dose). Each male is mated to 12 females over a 30 day period (2 females every 5 days) and then sacrificed.

Pathology studies are done on the reproductive organs, and levels of reproductive hormones (LH, FSH, androgens and testosterone) are measured in selected animals. Nine days after removal from the male, one female of each pair is autopsied and the number of implants and early and late resorption noted. The second female is allowed to deliver at term, the F_1 young counted, examined and weighed.

MAJOR FINDINGS AND PROPOSED COURSE: Completed protocols are not available for any compound since these are lifetime (18 month) studies and the contract was initiated 12 months ago. The following compounds have been put on test: diethylstillestrol (DES) (positive control), tetrachlorodibenzodioxin (TCDD), kepone, zearanol, mirex, DDE, 3,4,3',4' tetrachlorobiphenyl (4CB), 2,4,5,2', 4',5' hexachlorobiphenyl, (6-CB), and 2,4,5,2',4',5' hexabromobiphenyl. The fetotoxic and/or teratogenic dose has been established for each of these compounds in the CD-1 mouse. For example, zeranol, a synthetic mytoxin analog, was shown to cause skeletal abnormalities at a dose of 300 µg/kg/day on days 10-16 of gestation. The estrogenic potency of this compound was determined to be approximately 1/600 that of DES. In addition, 4CB was shown to be more fetotoxic than 6CB under similar experimental conditions. Studies on the postnatal reproductive effects of prenatally administered halogenated hydrocarbons, mycotoxins and other hormonally active xenobiotics will be continued. In addition, the potential for hormonal activity will be evaluated for other classes of environmental agents. Finally, localization of zearanol/zearalenone in specific fetal tissues will be performed by radioimmunoassay procedures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental chemicals can cause reproductive toxicity in mammals which is manifested in decreased fertility. However, these chemicals might affect male and female reproductive processes differently since oogenesis is an entirely prenatal event and spermatogenesis occurs throughout adult life in most eutherian mammals. The possibility exists that lesions of the reproductive tract which occur *in utero* might result in long term impairment of the reproductive tract in females and have a totally different effect on the male reproductive process.

The objective of the proposed study is to determine the differential effect of environmental chemicals on the reproductive capacity of male and female mice. Reproductive capacity is to be evaluated by the repetitive forced breeding method for females and by a serial mating technique for males.

These studies are done in such a way as to support existing programs in the Laboratory of Environmental Toxicology concerned with developmental toxicity and transplacental toxicity. The purpose of the contract is two-fold; to identify environmental chemicals that have potential developmental toxicity emphasizing reproductive tract lesions; and to identify chemicals to Intramural scientists that are toxic and need to be studied from a mechanistic viewpoint.

FISHERIES RESEARCH INSTITUTE - Univ. of Washington, Seattle, Wash.
(NIH-NO1-ES-7-2101)

TITLE: Carcinogenic Effects of Petroleum Hydrocarbons on Selected Marine and Estuarine Organisms

CONTRACTOR'S PROJECT DIRECTOR: Sam P. Felton and Bruce S. Miller

PROJECT OFFICERS (NIEHS): J. R. Bend, Ph.D., Visiting Scientist
Laboratory of Pharmacology
B. A. Fowler, Ph.D., Senior Staff Fellow
Laboratory of Environmental Toxicology

DATE CONTRACT INITIATED: December 16, 1976

CURRENT ANNUAL LEVEL: $92,556

PROJECT DESCRIPTION

OBJECTIVES: To compare incidence of tumors, particularly epidermal papillomas, in exposure and control groups of selected flatfish species in response to exposure to benzo (a) pyrene (BaP) and/or its electrophilic derivatives and to qualitatively examine the histopathology of comparable tumors occuring in both exposure and control groups.

METHODS EMPLOYED: Static and flowing sea water systems with aquaria and sand coated with BaP. Monitoring of BaP concentrations by gas chromatography, assay of aryl hydrocarbon hydroxylase (AHH), examination of fish tissue by routine histopathological procedures.

MAJOR FINDINGS AND PROPOSED COURSE: The first quarter efforts have been directed towards developing a suitable exposure system, optimizing AHH assay conditions for flatfish, refining analytical and biopsy procedures for histopathological assessment of fish tissues. These initial goals are in the process of completion; treatment of fish is expected to begin in the next quarter.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should provide fundamental information about the metabolism and biological responses to a carcinogenic petroleum hydrocarbon of marine organisms consumed as food. Results of the study should specifically yield useful data on the relationship between AHH activity and the carcinogenic susceptibility of marine organisms.

Proposed course is to expose flatfish to BaP and examine the relationship between AHH activity and carcinogenesis in these species.

MIDWEST RESEARCH LABORATORIES - Kansas City, Missouri 64141
NIEHS-72-2084

TITLE: Evaluation of the Environmental Toxicant, Vinyl Chloride Monomer

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): J. S. Woods, Ph.D, Head, Biochemical Toxicol. Group
Biochemical Toxicology Group, LET

DATE CONTRACT INITIATED: December 31, 1974

CURRENT ANNUAL LEVEL: $70,000

PROJECT DESCRIPTION

OBJECTIVES: This contract concerned the toxic effects of vinyl chloride monomer (VCM) and vinylidene chloride monomer (VDM) in mammals during low level, long term inhalation exposure. The specific objectives of these studies can be summarized as follows: (1) to determine nonmalignant biochemical and pathological alterations in specific organ functions of rats and mice during exposure to VCM and VDM, (2) to identify, innumerate and characterize malignant alterations occuring in VCM and VDM-exposed animals and to ascertain time-to-tumor development, and (3) to establish VCM and VDM response profiles for use in high to low dose, animal to human extrapolation studies.

METHODS EMPLOYED: Inhalation exposure techniques, conventional chromatographic procedures, U.V., visible and I.R. spectroscopy, histology, pathology, hematology, clinical blood chemistry, immunological techniques, and cytogenic analyses.

MAJOR FINDINGS AND PROPOSED COURSE: Vinyl chloride monomer and vinylidene chloride studies were initiated in September 1975. Rats and mice of both sexes have been exposed for 6 hours per day, 5 days per week to 50, 250, or 1000 ppm vinyl chloride or to 55 ppm vinylidene chloride. Hematologic, pulmonary, immunologic, biochemical, and cytogenic analyses conducted on exposed animal tissues have thus far demonstrated no adverse changes in non-malignant clinical laboratory parameters in rats of either sex and no apparent changes in these data in mice with the exception of increased pulmonary macrophage counts at 1000 ppm VCM in males. In contrast, numerous malignancies have appeared in both species after 12 months exposure to both VCM and VDM. In rats exposed to VCM hemangiosarcomas of the liver, lung, skin and adrenal gland, as well as tumors of the mammary gland and other sites were observed. Major tumors found in mice exposed to VCM included bronchiolar adenoma, hepatic hemangiosarcoma, various mammary gland tumors and malignant lymphoma. Tumor incidence was dose-related in most cases and was greater in females of both species. In animals exposed to VDM, one subcutaneous hemangiosarcoma of the skin was seen in rats, and 3 tumors of the lung and/or liver occurred in mice. Proposed studies are to continue the specified objectives of the contract in regard to the assessment of chronic toxic effects of VCM and VDM and to utilize these results in the design of models for the assessment of risk of exposure to these agents in human populations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These investigations will establish the effect and nature of chronic toxicities of selected environmental chemicals using rats and mice. This information will serve to form a rational basis for evaluation of the potential human health hazards produced by exposure to these chemicals.

PUBLICATIONS

Winston, J. M., Lee, C. C., Bhandari, J. C., Dixon, R. L., and Woods, J. S.: A study of the Carcinogenicity of Inhaled Vinyl Chloride and Vinylidene Chloride in Rats and Mice. Proceedings of the First International Congress on Toxicology (G. Plaa, ed.) Academic Press, NY, 1977 (in press).

NATIONAL MARINE FISHERIES SERVICE - Beaufort, North Carolina 28516
(Interagency Agreement Between NIEHS & NOAA)

TITLE: The Physiological Effects of Arsenic, Cadmium, and Copper on Marine Shellfish

CONTRACTOR'S PROJECT DIRECTOR: David W. Engel, Ph.D.

PROJECT OFFICER (NIEHS): B. A. Fowler, Ph.D., Senior Staff Fellow
Biochemical Toxicology Group, LET

DATE CONTRACT INITIATED: July 23, 1975

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: This study was undertaken to determine the physiological, biochemical, and ultrastructural effects of arsenic, cadmium, and copper on tissues of marine shellfish in comparison with those previously observed in mammals. Large numbers of shellfish will be exposed to these elements for subsequent incorporation of freeze-dried shellfish meats into rat diets for evaluation of pharmacokinetic and toxicological differences in comparison to similar doses of these metals administered in drinking water.

METHODS EMPLOYED: Flowing seawater systems with continuous trace element injection, atomic absorption spectroscopy, radiotracer analyses, histological and ultrastructural techniques, cellular respiration studies.

MAJOR FINDINGS AND PROPOSED COURSE:

Effects of Copper on Shellfish. The effects of varying copper concentrations on shellfish gill tissue respiration rates were studied. Four species of lamellibranch mollusks were used in the determinations: the American oyster, Crassostrea virginica; the hard clam, Mercenaria mercenaria; the bay scallop, Argopecten irradians; and the ribbed mussel, Modiolus demissus. The excised gill tissue from the four species of mollusks exhibited species specific responses to cupric ion concentrations between $10^{-6.6}$ M and $10^{-8.1}$ M. Respiration rates of scallop and clam tissue increased significantly with increased cupric ion concentrations. Gill tissue from the mussel showed smaller increases in respiration than the scallop or clam tissue, and the respiration rates of oyster gill tissue were not significantly affected by the presence of copper in these ranges. Other studies were conducted on oysters exposed to flowing natural seawater containing copper at 0.1 ppm as copper chloride for 14 days. Samples were taken at 1, 3, 7 and 14 days. The accumulated copper in the oyster gill tissue significantly affected the tissue respiration rate by the 14th day. There was a steady increase in the respiration rates and copper concentrations of the treated oyster tissue relative to the control tissue from the first through 14th day. Ultrastructural examination of columnar epithelial cells from these animals disclosed increased numbers of dense, lysosome-like bodies which were found to contain copper and iron by x-ray microanalysis.

Effects of Cadmium on Shellfish. Another series of experiments were conducted to determine the effects of salinity on cadmium toxicity and availability, using the grass shrimp, Palaemonetes pugio, as the test organism. To test for toxicity, the shrimp were exposed to 1.0, 0.5, 0.25, 0.125 ppm and 0.1 ppb of cadmium at salinities of 4, 8, 16 and 28 °/oo. Exposures were made in 4-liter polyethylene tubs for 96 hours. Measurements of cadmium availability and uptake were made using ^{115m}Cd at salinities similar to those used for the toxicity tests. Uptake of the radionuclide by grass shrimp was measured every 24 hours for a period of 96 hours using liquid scintillation counting techniques.

The results of the cadmium exposures indicated that toxicity with respect to total metal concentration was salinity dependent with the greatest toxicity observed at the lowest salinity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will provide a basis for evaluating mechanisms of uptake and cellular responses to chronic low level trace metal exposure in tissues of relatively simple organisms in comparison to those observed in mammals.

Data generated from this effort should also prove useful in evaluating differences in trace metal pharmacokinetics and toxicity following ingestion of metal contaminated marine shellfish.

The future course of the project will involve the specified objectives of this interagency agreement in relation to understanding the subcellular mechanisms of trace element uptake and toxicity in marine organisms in comparison to mammals. This arrangement also provides a mechanism for estimating potential human risk following ingestion of metal contaminated marine shellfish.

PUBLICATIONS

Engel, D. W. and Fowler, B. A.: Accumulation and biological effects of copper and cadmium on marine shellfish. Proceedings of the technical review of the Interagency Marine Research Program associated with energy development. (in press).

OAK RIDGE NATIONAL LABORATORY, U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION - Oak Ridge, Tennessee 37830
(ERDA 40-524-75)

TITLE: Environmental Teratology Information Center (ETIC)

CONTRACTOR'S PROJECT DIRECTOR: John S. Wassom, B.Sc.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: August 15, 1975

CURRENT ANNUAL LEVEL: $230,000

PROJECT DESCRIPTION

OBJECTIVES: To establish a teratology information center.

METHODS EMPLOYED: (1) To collect reprints of all publications that contain data pertinent to determination of the teratogenic potential of environmental agents or conditions to which man is exposed; and (2) to use facilities available at Oak Ridge National Laboratories to extract specific blocks of information from the relevant references and develop a method permitting computer storage and retrieval of this information.

MAJOR FINDINGS AND PROPOSED COURSE: Initial effort was concentrated on accumulation of bibliographic information from studies conducted on warm-blooded animals, whether the results were positive or negative. This task is well underway as over 7500 reprints have been identified and processed. The information collected is stored on magnetic tape in a form that can be readily accessed by the National Library of Medicine for inclusion in TOXLINE. The information is indexed by agent, ETIC access number, Chemical Abstract Service Registry Number, Wisswesser Linear Code, test animal, and investigator. A chemical dictionary has been developed that lists all agents in the system and provides molecular formulas, synonyms, trade names, and generic names.

Current studies are located by personal contact with teratologists, by manual scanning of about 30 key journals, and by automated scanning of available computer tapes from large multidisciplinary data bases (e.g., Chemical Abstracts) via a teratology profile. Earlier studies are located through use of teratology files made available by individuals and organizations.

Names and addresses of members of the American, European, and Japanese Teratology Societies have been entered on tape; hopefully contact with these scientists will facilitate aquisition of new information to be added to the system.

The advisory committee selected to guide the development of ETIC was expanded to three specialists. This committee continues to provide guidance to the Project Officer in the development of ETIC. It was at their suggestion that experts from the National Bureau of Standards made a formal analysis of information flow at ETIC/Oak Ridge, an analysis which provided suggestions for

improvement, and identified advances in technology that should be applied. Although ETIC will not be open for business officially until it is available on TOXLINE, information on more than 534 agents has been given to more than 120 questioners to date.

The proposed course is to have the ETIC system placed on TOXLINE, to continually update input of the first six fields of information, to include information from non-published studies to be found in government agencies as well as in private industry, to extract more information from each article, and to develop a mechanism for providing user access to the additional information.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Teratology data on chemical and other agents are widely scattered in the literature, and there is currently no central file listing agents which have been tested, let alone to what extent, or in what species. Teratology data are often included in publications on general toxicity and the chemicals tested often do not appear in titles, hence data are very difficult to locate. This information is necessary for those responsible for assigning priorities for study and for evaluating the teratogenic potential of individual agents. Creation of ETIC will permit ready access to the references containing teratology on each agent and will provide an extract of the pertinent information contained. Through use of ETIC, unplanned duplication of published studies can be prevented and it is planned that names of investigators with studies in progress on given agents will be listed.

RESEARCH TRIANGLE INSTITUTE - Research Triangle Park, NC 27709
(NO1-ES-6-2127)

TITLE: Teratogenic Potential of Environmental Agents

CONTRACTOR'S PROJECT DIRECTOR: Gary Kimmel, Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: June 1, 1976

CURRENT ANNUAL LEVEL: $255,026

PROJECT DESCRIPTION

OBJECTIVES: To determine the teratogenic potential in animals of environmental agents.

METHODS EMPLOYED: Standard teratology techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Positive teratology was obtained following oral administration of symmetrical polychlorinated biphenyls to mice from days 6 through 15 of gestation. Correlations are sought between the teratogenicity found and chemical structure or other chemical or physical properties of these PCB isomers.

Mice were given either tap water or purified water to drink during pregnancy to determine whether impurities present in chlorinated tap water contribute to the background incidence of resorptions or malformation seen among the offspring. Purification of water by demineralization and removal of organic impurities did not significantly decrease the incidence of fetal death or malformation.

The proposed course is to complete current studies publish the results, and test additional agents. Agents currently being considered include glutaraldehyde, and methyl bromide.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most environmental agents have not been tested for teratogenic potential. This contract was established to provide capability to test agents of high priority either because of possible exposure to man or because the information obtained can be used to aid our understanding of how human development is altered by them.

LABORATORY OF PHARMACOLOGY

LABORATORY OF PHARMACOLOGY
Summary Statement

The Laboratory of Pharmacology initiates and carries out research programs that are primarily concerned with (1) mechanisms by which environmental pollutants and effectors cause toxicity, (2) the development of better test systems for detecting and evaluating early toxic effects of chemicals occurring as environmental contaminants, and (3) extrapolating from animal test systems across species and to man. The Laboratory is organized into three formal sections and one work group. The sections are Toxication-Detoxication, Marine Pharmacology and Biomedicine, and Pharmacokinetics; the work group is Molecular Pharmacology and Biomedicine.

Program

Toxication-Detoxication Section: This program area is concerned with the role of chemical metabolism in the production of tissue damage and toxicity. Metabolism leading to increased toxicity may be balanced by reactions with detoxifying systems. This balance between toxication and detoxication pathways may be altered by pollutants, other chemicals, pathologic states, physiological changes (age or pregnancy), species and strain differences, hormonal changes, or it may be different in one tissue from another. Chemical-metabolizing systems have been or are being characterized in tissues most likely to contact the environment, i.e., skin, intestine, lung, and liver, as well as in steroidogenic tissues containing germinal cells (ovary, testis). Characterization includes substrate and inhibitor specificity, ability to be activated, induced or inhibited by exposure to other chemicals and changes in physiological status (e.g., pregnancy), and measurement and purification of multicomponent systems or enzyme families (e.g., cytochrome P-450-dependent mixed-function oxidase [MFO] systems or the glutathione S-transferases).

Two different forms of cytochrome P-450 (absorption maxima of reduced-CO bound cytochrome occurred at 425.5 nm and 450 nm, respectively) were separated from the same rabbit lung microsomal preparation. The lipid requirement for maximal MFO activity is being studied in reconstituted systems. Cytochrome P-448 was purified from livers of control and 3-methylcholanthrene pretreated rabbits; the cytochromes were identical based on purification procedures, monomeric molecular weight, and immunological tests.

Techniques are being developed to separate and culture lung cells for studying chemical toxication and detoxication mechanisms at the cellular level.

Mammalian skin has very low, but detectable, MFO activity. In mice, at least, this activity seems to be increased following exposure to shortwave UV irradiation (254 nm) or to a sunlamp -- conditions that might mimic prolonged exposure to sunlight.

The effects of pretreating mice, rats, guinea pigs, and rabbits, with known inducers of hepatic MFO activity, on intestinal xenobiotic-metabolizing enzyme activities were studied. The induction of intestinal MFO enzymes varied with the animal species and the xenobiotic used as substrate, which is consistent with the idea that chemically induced toxicity in this tissue may be partially dependent on species.

The formation and subsequent biotransformation of chemically reactive alkene and arene oxides, formed from alkenes and polycyclic aromatic compounds, are being studied in liver and several extrahepatic tissues of rats and rabbits. Comparative studies utilize partially purified enzymes, subcellular fractions of tissue homogenates and isolated perfused lung, liver, and testis preparations. The relative importance of glutathione S-transferase and epoxide hydrase activities in the metabolism and detoxication of chemically different epoxides is being assessed. Substrate specificities with different synthetic oxides that are thought to be carcinogens and/or mutagens are tested. The effect of pre-exposure of animals to other xenobiotics, to depletion of required endogenous cofactors, and to normal physiological control mechanisms on enzyme activities and epoxide-induced toxicity is also determined.

All of the studies in this section seek to understand the relationships between xenobiotic metabolism and the localized toxicity (i.e., target organ), both acute and chronic, caused by exposure to environmental pollutants.

Marine Pharmacology and Biomedicine: This program area conducts studies on uptake, distribution, metabolism, and excretion of pollutants by various marine species, the role of metabolism of these pollutants in their storage and the chemical moiety stored in these species, and the use of marine organisms to identify those physiological processes that are especially sensitive to exposure to certain xenobiotics (e.g., organochlorines).

One area of major emphasis is the potential accumulation of carcinogens, mutagens, teratogens, cytotoxins, and marine toxins (i.e., chemicals that may be particularly dangerous to man) in aquatic species. The effect of exposure to pollutant mixtures on accumulation of chemicals is also studied.

Operation of a year-round facility (the C.V. Whitney Marine Laboratory, University of Florida, St. Augustine) allows investigations which expand and complement our seasonal (summer) studies at Mount Desert Island Biological Laboratory, Maine. Comparisons are being made of the effect of environmental temperature on metabolic, excretory, and storage processes for pollutants. Currently, the disposition of aliphatic and aromatic constituents of crude and refined oils is under investigation.

The biotransformation of various organic pollutants is studied in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Particular emphasis is placed on studying formation and further metabolism of chemically reactive electrophilic metabolites. Both cytochrome P-450-dependent microsomal mixed-function oxidases and alkene- or arene oxide-metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control marine species and in species pre-exposed to environmental contaminants such as the polycyclic aromatic

hydrocarbons. Where biologically significant induction is observed, the induced system is being characterized in considerable detail, e.g., in little skates, where cytochrome P-448 has been identified for the first time in an aquatic animal.

The pharmacokinetics of pollutant distribution in selected marine species is being determined and compared with mammals (in collaboration with the Pharmacokinetics Section). So far we have shown that a polychlorinated biphenyl (PCB) isomer is stored in different tissues in different marine species and that the biological half-life of PCB correlates with the ability of the species to metabolize it. Compared with mammalian systems, marine species have a relative deficit in the capability to carry out oxidative chemical metabolisms, while possessing adequate levels of enzymes for conjugation reactions. Thus, we have shown that water-soluble pollutants, such as 2,4-D and 2,4,5-T, are rapidly conjugated with amino acids and excreted in the bile and urine of teleosts and elasmobranchs.

Other areas being investigated include the importance of renal and hepatic organic anion transport systems in elimination of xenobiotic metabolites by marine species, the role of intracellular binding proteins (the glutathione S-transferases) in cellular transport and toxicity of organic anions, and the role of membrane function in relationship to target organ accumulation of xenobiotics or their metabolites and toxicity. Emphasis is placed on correlating in vitro biochemical and physiological measurements with in vivo function (e.g., osmoregulation). Since many endogenous compounds utilize active transport systems in various tissues of the body, these studies also attempt to elucidate interactions between xenobiotic metabolites and endogenous molecules utilizing the same transport mechanisms.

The Section is also involved in developing a national program in marine pharmacology and biomedicine as directly applicable to human health problems (models for human disease, sources of toxic materials for man). The main direct concern to the NIEHS will be to provide a focus for DHEW activities in marine biomedical studies.

Molecular Pharmacology and Biochemistry Work Group: This group is involved in studies which can be categorized as pharmacological and biochemical research on synthesis, metabolism, release, isolation, and identification of endogenous chemicals (lipids, proteins, enzymes) involved in lung function, both in normal and in disease states. Information is being used to develop markers for early human disease states of possible environmental origin.

One group is concerned with the identification and characterization of components which are lung specific or pulmonary disease-related which could be used as diagnostic markers for pulmonary damage or disease. Current attention is being focused on: (1) the soluble alkaline phosphatases of the normal lung, 2) abnormal hydrolases present in pulmonary lavage effluents from patients with alveolar proteinosis, and 3) enzymic components of human amniotic fluid which originate in the fetal lung.

Human lung was found to contain two distinct alkaline phosphatases having molecular weights of 213,000 and 125,000 daltons. These enzymes appear to be lung-specific with the lower molecular weight form present in extracellular lining of airways. This work was done in collaboration with Duke University and George Washington University. Further studies have characterized the high molecular weight (> 20 x 10^6 daltons) soluble alkaline phosphatase (AP) complex found in pulmonary lavage effluents of patients with alveolar proteinosis, a disease of suspected environmental etiology. After appropriate chemical treatment of the complex, a low molecular weight AP is released which appears similar to the AP's found in normal lung.

Another group of investigators is focusing on the potential of normal and neoplastic cells of the lung to synthesize polypeptide hormones. A pituitary-like cell has been identified in the lungs of several species and designated P cell. These cells may be target progenitors for lung tumors, such as oat cell carcinoma, which are capable of producing bioactive polypeptide hormones. Four mammalian squamous cell carcinomas were found to contain immunoreactive adrenocorticotropic hormone (ACTH) and this immunoreactivity was associated with "big ACTH" rather than "small ACTH.: "Big ACTH" was also found in the extract of bovine pituitary gland.

Since human bronchogenic squamous cell carcinomas are known to produce a large form of ACTH, an investigation has been initiated to purify this material from bovine pituitary glands so that a specific radioimmunoassay might be developed. Hypersecretion of cell-specific or tumor-specific polypeptides or proteins could serve as a means for sensitive detection of pulmonary neoplasia or preneoplasia.

A third group of investigators is studying the biosynthesis, release, and metabolism of prostaglandins (PG) and thromboxanes (TX) in pulmonary tissue. A transport system responsible for removing PG from the circulation into the lung was observed and characterized by the group. The structural requirement necessary for transport was examined using the isolated perfused rat lung. Some chemicals and anti-inflammatory agents inhibited the transport system. Exposure of rats to pollutants (O_2, NO_2) inhibited the pulmonary metabolism of PG but did effect the transport system. The major metabolites of PG, 15-keto PG were found to be further metabolized by glutathione S-transferase to the corresponding glutathione conjugate.

PG and TX biosynthesis were studied. During the formation of PG and TX, a metabolite(s) of arachidonic acid (AA), the precursor substance for PG, was covalently bound to tissue protein. Since AA or the known end-products, PG and TX, did not react with tissue, reactive intermediates were probably formed which reacted with tissue. The nature of the reactive intermediate is currently under investigation. The covalent binding of these endogenous chemicals may be associated with or be responsible for pulmonary toxicity induced by environmental agents. Irritation of pulmonary tissue by exposure to pollutants initiates the biosynthesis and release of PG and TX. Covalent binding of AA metabolites was found in other tissues including platelets, seminal vesicles, kidney, and stomach.

These investigators also studied the co-oxygenation of chemicals during the formation of PG and TX. During this formation in guinea pig lung, benzpyrene (BP) was oxidized to metabolites which appeared to be quinones with little or no formation of monohydroxy- and dihydro-diol metabolites, but in the presence of NADPH, the major benzpyrene metabolite was dihydrodiol. Approximately 20% of the BP oxidized during the formation of PG was covalently bound to protein while only 5-8% of the metabolites formed in the presence of NADPH was bound to protein.

Activation of benzpyrene during the formation of PG and TX may be related to the induction of pulmonary carcinogenesis and may represent an alternate mechanism to oxidation of chemicals by the cytochrome P-450 system.

Pharmacokinetics Section: This section is conducting research in at least two major categories: (1) whole body distribution of pollutants and formulation of pharmacokinetic models for extrapolation, and (2) uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs, with present emphasis on lung and liver. The kinetics of chemical disposition in intact animals and isolated organs are being determined in order to construct mathematical models of these processes which can be used: (a) to better define and understand rate-limiting steps in the process, (b) to extrapolate distribution profiles of chemicals from one tissue to another, one species to another, and to man, and (c) to predict tissue storage of pollutants from knowledge of routes, dose, and number of exposures. Comparisons of the kinetics of pollutant distribution in mammalian vs. marine species are being carried out in collaboration with Marine Pharmacology and Biomedicine, as previously mentioned.

Through extensive collaboration with the Biomedical Engineering and Instrumentation Branch, DRS, NIH (Drs. R. L. Dedrick and R. Lutz), pharmacokinetic models have been developed for four PCB isomers in the rat. The biochemical and physiological parameters used in these models were determined from studies in bile-cannulated animals, isolated perfused liver studies, and whole animal disposition studies.

These models can predict the disposition of the PCBs after a single i.v. or oral dose. Recently the models have sucessfully predicted the disposition of PCBs in rats receiving multiple doses for six weeks. The pharmacokinetic models developed for rats were modified to enable extrapolation to the mouse. Model parameters such as tissue values, flow rates, and gut transport were adjusted in accordance with animal weight and the predicted PCB disposition compared to experimental data. Tissue and excreta concentration time curves obtained in experimental animals generally agreed with model predictions. The species independence of the pharmacokinetic model will be tested in dog and monkey. Experimental data will be obtained from a contract with the University of Arizona.

The disposition of two additional PCB isomers (3,3',5,5'-PCB and 2,2',3,3',5,5'-PCB) was studied in rat to further examine the influence of degree and position of chlorination on disposition of PCB. The 4-CB, which does not have adjacent unsubstituted carbons, was metabolized more slowly than predicted from the degree of chlorination. The 6-CB, which has an adjacent unsubstituted carbon,

was metabolized as rapidly as the 1-CB previously studied. The metabolites of various PCBs were isolated and identified as monohydroxy- and dihydroxy-diol metabolites. Metabolism of PCBs appears to be a prerequisite for excretion. PCBs that are persistent in the body are those isomers that are slowly metabolized by animals.

The disposition of the major component of Firemaster BP-6^{R} (2,4,5,2',4',5'-hexabromobiphenyl) was studied in rats. The polybrominated biphenyl appears to have similar pharmacokinetic properties to the corresponding hexachlorobiphenyl and the half-life of the hexabromo isomer was longer than the corresponding hexachlorobiphenyl isomer. A pharmacokinetic model for hexabromobiphenyl is being developed.

The disposition of Kepone, a halogenated insecticide, was also studied. Kepone was stored primarily in the liver and excreted in the bile. The measured half-life of 18 days was due primarily to reabsorption of Kepone from the gut after excretion in the bile.

Another continuing major effort of the Section is in examination of pharmacokinetic functions of the lung with regard to both exogenous and endogenous chemicals. These studies involve the use of the isolated perfused rat, guinea pig, and rabbit lung preparations as research tools. With regard to exogenous chemicals, detailed models were developed for the dynamic interaction of the perfused lung with circulating chemicals. With regard to endogenous chemicals, a pharmacokinetic model was developed for the prostaglandin detoxification system. From this model, it was evident that flow rates are important in examining transport systems in isolated organs. In general, elucidation of the rates and mechanisms of lung uptake, storage, and metabolism of chemicals may allow prediction of possible toxic interactions between environmental pollutants and other chemicals, including drugs and endogenous chemicals such as prostaglandins.

The major effort of the year was devoted to modification of existing perfused lung techniques and development of new methods to examine the uptake and metabolism of inhaled environmental pollutants. Several environmental carcinogens, e.g., benzpyrene, have been selected for study and attempts will be made to correlate the uptake and metabolism of these chemicals to the development of lung tumors.

Personnel

Two major personnel shifts took place during the year. Dr. L. G. Hart (Acting Laboratory Chief) moved into the position of Assistant to the Scientific Director, NIEHS, about midyear, and Dr. J. R. Fouts (Scientific Director) assumed the responsibility of Acting Chief. A group headed by G. E. R. Hook transferred from the Molecular Pharmacology and Biochemistry Work Group into the Laboratory of Environmental Toxicology later in the year. Other members of the group include Dr. J. W. Spalding (Research Biologist), Dr. D. Nadeau (Visiting Fellow), Dr. D. Bell (Guest Worker), and Ms. L. Gilmore (Biologist).

Additions to the Laboratory were: Research Physiologist -- J. B. Pritchard (with Dr. Bend); Research Chemist -- L. Lazarus (with Dr. DiAugustine); Research Biologist—J. W. Spalding (transferred from LET, with Dr. Hook); Staff Fellow -- W. Colburn (with Dr. Eling); Visiting Associates -- A. Wilson (with Dr. Eling), H. Mukhtar, T. Elmamlouk (with Dr. Bend); Visiting Fellows -- O. Hernandez, R. Weatherby (with Dr. Bend), M. Szutowski, C. R. Wolf (with Dr. Philpot); D. Crutchley, A. Kung (with Dr. Eling), L. Ball (with Dr. Chhabra), M. Khan (with Dr. DiAugustine), J. Leakey (with Dr. Fouts); NIH Postdoctoral Fellow -- J. Maguire (with Dr. Bend); Intergovernmental Personnel Act -- G. Neufeld (with Dr. Bend), D. Bell (with Dr. Hook).

Losses from the Laboratory included: J. Maguire (NIH Postdoctoral Fellow), M. Szutowski (Visiting Fellow), O. Hernandez (Visiting Fellow, transferred to EBCB), M. Reasor (NIH Postdoctoral Fellow), J. Brittian (technician).

Other Activities

Dr. J. R. Bend: Member, Editorial Advisory Board for Drug Metabolism and Disposition; Visiting Scientist -- C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; Member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; invited participant in a symposium entitled "Formation of Chemically Reactive Metabolites As a Cause of Drug Toxicity," sponsored by ASPET, New Orleans, La., 1976; invited participant at a conference on "Aquatic Pollutants and Biological Effects with Emphasis on Neoplasia," sponsored by the New York Academy of Sciences, Sept. 1976; invited speaker at a symposium entitled "Status of Predictive Tools in Application to Safety Evaluation: Present and Future," sponsored by NIH and NCTR, Little Rock, Ark., 1976; invited speaker on "Metabolic Reactions In Vivo and In Vitro" in conference on "Short Term Tests for Carcinogenicity," sponsored by Toxicology Forum, Hunt Valley, Md., Feb. 1977; invited lecturer at the NATO Workshop on Ecotoxicology, University of Surrey, Guildford, England, July 11-Aug. 5, 1977; invited speaker at symposium entitled "Environmental Pharmacology of Lung," sponsored by ASPET, Columbus, Ohio, Aug. 1977; presented seminar entitled "Metabolism of Xenobiotics by Marine Species," Department of Pharmacology, University of Wisconsin, Dec. 1976.

Dr. R. S. Chhabra: Member of the Library Committee, NIEHS; invited speaker to International Symposium on "Nutrition Drug Interactions, Ames Iowa, 1976.

Dr. R. P. DiAugustine: Adjunct assistant professor, Department of Medicine, Duke University; invited lecturer at Department of Pathology, Duke University; member of NIEHS Medical Sciences Lectures Committee; invited speaker at American Thoracic Society (Eastern Section), Oct. 1976.

Dr. T. E. Eling: Adjunct assistant professor, Department of Medicine, Duke University; invited speaker at Deer Lodge Conference on clinical pharmacokinetics; invited speaker at Gordon Conference on drug metabolism. Reviewer for Drug Metabolism and Disposition, Life Sciences, Cancer Research, Journal of Pharmacology and Experimental Therapeutics.

Dr. L. G. Hart: Adjunct associate professor, Department of Pharmacology, University of North Carolina, lectures presented to medical and graduate students of University of North Carolina; member of NIEHS Safety Committee; NIEHS project director for coordinating efforts in three task areas under the "health effects of the non-nuclear energy technologies" program; associate managing editor (U.S.P for Chemico-Biological Interactions; editorial board member of Environmental Health Perspectives; chaired symposium on "Pesticides in Aquatic Environments," 15th Congress of Entomology, Washington, D. C.

Dr. G. E. R. Hook: Adjunct assistant professor, Department of Medicine, Duke University; co-managing editor and editorial board member of Environmental Health Perspectives; editoral board member of

Dr. R. M. Philpot: Adjunct assistant professor, Department of Entomology, North Carolina State University; member of NIEHS editorial board; editor, Chemico-Biological Interactions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80001-06 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Microsomal Mixed-Function Oxidase Systems: Specificity and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Philpot	Research Chemist	LP NIEHS
Other:	M. Szutowski	Visiting Fellow	LP NIEHS
	C. R. Wolf	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.25	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO) from various sources. Present work involves the purification of cytochrome P-450, NADPH-cytochrome c reductase, and lipid fractions from rabbit pulmonary and hepatic microsomes. The cytochrome P-450s are being examined by UV-visible spectroscopy, electron paramagnetic resonance spectroscopy, SDS-gel electrophoresis, and by activities in reconsituted systems. The long-range objective of this work is to determine the influence of: 1) multiple forms of the enzymic components of the MFO system; 2) endogenous compounds; and 3) exogenous compounds (inhibitors and inducers) on the substrate specificities of MFO systems from different tissues and species.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The components of the liver mixed-function oxidase (MFO) systems are solubilized and separated by the methods developed by Lu and co-workers. These methods have been modified for application to the rabbit pulmonary MFO system. Pulmonary cytochrome P-450 is being purified by the combination of existing techniques for liver cytochrome purification and the development of new techniques. Spectral analysis of cytochrome fractions is carried out according to methods developed in this laboratory.

MAJOR FINDINGS AND PROPOSED COURSE:
1) The Rabbit Pulmonary MFO System. Two forms of cytochrome P-450 have been partially purified (about 40-fold) from rabbit pulmonary microsomes. The spectra of the reduced cytochromes combined with carbon monoxide differ: form I has a maximum absorbance at 452.5 nm and form II at 450 nm. Both forms of the cytochrome, as well as NADPH-cytochrome c reductase, can be separated and recovered from the same microsomal preparation. This is accomplished by chromatography of detergent-digested microsomes on DEAE-cellulose using Emulgen 913 and a gradient of KCl as eluants. Pulmonary MFO activity has been reconstituted by combining the cytochrome and reductase components from the lung. The lipid requirement for maximum activity in the reconstituted system includes both the phospholipid and neutral lipid fractions obtained from pulmonary microsomes.

2) The Rabbit Hepatic MFO System. Cytochrome P-448 has been purified to apparent homogeneity from 3-methylcholanthrene (3-MC)-treated and untreated rabbits. The cytochromes from either source can be purified by the same methods, have the same monomeric molecular weights, and are immunologically identical. Rabbit hepatic cytochrome P-448 exists in the "high spin" configuration but can be converted to the "low spin" state by detergents, butanol, or caffeine. Conversion back to the "high spin" state is accomplished by removing the added ligand. Thus, if the cytochrome is in the "high spin" state due to a bound endogenous compound then the compound is not readily removed from the protein moiety of the cytochrome. Bound 3-MC is not responsible for the "high spin" configuration of cytochrome P-448 purified from hepatic microsomes of 3-MC-treated rabbits.

The present work involving the solubilization, reconstitution, and determination of substrate specificities of the MFO systems from rabbit liver and lung will be continued. The effect of 3-methylcholanthrene in rat and rabbit will be further examined in an effort to identify the factors responsible for the differences noted in responses. Efforts to purify cytochromes from the two tissues will continue. Immunological studies are being initiated with Dr. Faith (EBCB) and EPR studies with Dr. Rajagopalan (Duke University).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of MFO systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environment pollutants, and pesticide detoxification are to be fully comprehended.

This problem is complicated by the multitude of functional differences and responses exhibited by MFO systems from various sources. Identification of the factors which control the activities of MFO systems is essential to our understanding and manipulation of this complex biological process.

The methods by which the lung deals with inhaled substances are of particular importance. The lung is a target organ for the action of polycyclic hydrocarbons which are an important class of environmental pollutants. It has been hypothesized that these compounds must be metabolized by the MFO system in order to become effective as carcinogens and/or mutagens. The differences between the lung and liver systems in dealing with these compounds may provide part of the explanation for target organ specificity.

PUBLICATIONS

Philpot, R. M. and Arinc, E.: Separation and purification of two forms of hepatic cytochrome P-450 from untreated rabbits. Molec. Pharmacol. 12: 483-493, 1976.

Arinc, E. and Philpot, R. M.: Preparation and properties of partially purified pulmonary cytochrome P-450 from rabbits. J. Biol. Chem. 21: 3213-3220, 1976.

Philpot, R. M. and Arinc, E.: Solubilization, separation, and partial purification of cytochrome P-450 and cytochrome b_5 from hepatic microsomes of the little skate, Raja erinacea. Bull. Mt. Desert Island Biol. Lab. In press, 1976.

Arinc, E. and Philpot, R. M.: Catalytic hydroxylation activities and some other properties of partially purified rabbit pulmonary cytochrome P-450. The Third International Symposium on Microsomes and Drug Oxidations. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80002-07 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. R. Devereux	Research Biologist	LP NIEHS
	J. R. Fouts	Scientific Director	NIEHS
Other:	G. E. R. Hook	Senior Staff Fellow	LP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the long range purpose of this project to study how various chemicals and physiological changes affect xenobiotic metabolism by the body. This laboratory has concentrated its effort on the lung as a target organ for exposure to environmental stresses. Present studies include isolation and culture of rabbit lung cell types for the purpose of studying localization of xenobiotic metabolism within the lung and toxication-detoxication mechanisms in individual cell populations. The following model enzyme systems are being set up for study of individual drug metabolism pathways in cell populations: p-xylene hydroxylation (cytochrome P-450 dependent), benzpyrene hydroxylation (cytochrome P-448 dependent), and N,N-dimethylaniline N-oxidation (cytochrome P-450 independent). Cells which have drug-metabolizing activity will be characterized and the cellular metabolic activity compared to that of microsomes prepared from lung tissue. Mechanisms of toxication and detoxication in lung cells will be studied in order to further understand the nature of this nonrespiratory function of the lung.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various enzyme preparations, including trypsin, pronase, and elastase, have been used for dispersal of lung cells. Populations of cells are separated from the cell digest according to their rates of sedimentation by the technique of centrifugal elutriation. Other methods employed for cell separation include density gradients and differential attachment to tissue culture plates. Cells are studied using light microscopic (including fluorescence microscopy) and electron microscopic techniques. Spectrophotometric, fluorimetric, and radiometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques are being developed to disperse and separate the many lung cell types in order to localize and study drug metabolism in individual cell populations. Some success has been achieved with the use of pronase digestion followed by cell separation in the elutriator centrifuge. Major efforts are now being undertaken to improve cell yields as well as to start cell cultures of individual populations by the techniques of cloning and differential attachment to tissue culture plates. Future research will focus on the study of toxication and detoxication mechanisms in order to understand the ways chemical and physiological stresses alter these systems within isolated cell populations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: This research project seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Use of lung cells to study drug metabolism may lead to a better understanding of lung damage and repair mechanisms. Comparisons will be made of cellular versus microsomal and isolated perfused lung xenobiotic metabolism systems to see where differences exist and what this may contribute to understanding toxication and detoxication mechanisms in the body. Studies of changes caused by steroids, metal ions or other chemicals, or physiological changes (age) on these cellular systems may aid our understanding of when the body is more susceptible to toxic agents, and, on a cellular basis, how the body handles these insults. Studies of xenobiotic metabolism in lung cell populations may give us a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals and physiological stresses can alter these systems and this balance.

PUBLICATIONS

Harper, C., James, M. O., Devereux, T. R., Patel, J., Bend, J. R., and Fouts, J. R.: Characteristics and development of drug metabolism by pulmonary microsomes. Agents and Actions 6: 527-530, 1976.

Devereux, T. R. and Fouts, J. R.: The effect of dexamethasone treatment on N,N-dimethylaniline demethylation and N-oxidation in pulmonary microsomes from pregnant and fetal rabbits. Biochem. Pharmacol. In press.

Devereux, T. R., Philpot, R. M., and Fouts, J. R., The effect of Hg^{+2} on rabbit hepatic and pulmonary solubilized, partially purified N,N-dimethylaniline N-oxidases. Chem.-Biol. Interact. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-04 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Xenobiotic-Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Pohl	Research Biologist	LP NIEHS
	J. R. Fouts	Scientific Director	NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The project is designed to elucidate the role of xenobiotic-metabolizing enzymes in skin as mediators of toxicity of environmental agents. Mixed-function oxidase (including aryl hydrocarbon hydroxylase), glutathione S-epoxide transferase, UDP-glucuronyl transferase, and L-histidase activities are measured in pieces of whole skin or epidermis, or in microsomes or cytosol from skin of hairless mice (Hrs/J) after exposure of the mice to ultraviolet radiation, polycyclic hydrocarbons, chlorinated hydrocarbons, etc. The wavelength of maximum absorbance in the carbon monoxide spectra of reduced cytochrome P-450 is identified after separating the cytochrome from contaminating mitochondrial pigments.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Whole skin is chopped into 1 mm pieces with a McElwain tissue chopper and aliquots are weighed for assay of mixed-function oxidase (MFO) activity. In some experiments whole skin or epidermis is disrupted with a Polytron cell disruptor before microsomal and cytosol fractions are isolated by standard homogenization and ultracentrifugation techniques. Assays of fluorescent metabolites of 7-ethoxycoumarin and 7-ethoxyresorufin were modified by organic solvent extraction procedures to increase sensitivity of the assays.

MAJOR FINDINGS AND PROPOSED COURSE: Female hairless mice (Hrs/J) were used in the following studies.

7-Ethoxycoumrain deethylase (7-EC) activity measured in chopped whole skin from mice exposed to a single shortwave UV irradiation (λ 254 nm) was elevated up to 2-fold after 3 hrs, the effect lasting at least 24 hrs. A single exposure to sunlamp (g 280 nm thru visible) at the minimum erythemal dose increased activity less than 50% at time periods from 15 min to 5 days after exposure. When either sunlamp or shortwave UV radiation was administered 24 hrs after topical application of Arochlor 1260 (2 mg/mouse), there was no increased 7-EC activity 2 hrs after exposure. Arochlor 1260 alone did not increase activity, but these experiments revealed a 40% increase in activity in mice treated with 0.1 ml of vehicle (DMSO or acetone) alone, compared with untreated controls. When exposure to shortwave UV radiation was repeated 1, 3, 5, and 6 days after Arochlor 1260 treatment, 7-EC and 7-ethoxyresorufin deethylase (7-ERF) activities in mouse skin were increased 4-fold over acetone-treated controls, although activities in skin from mice treated with Arochlor 1260 alone were not increased and were elevated only 50% in skin from mice that received UV radiation (after acetone). UDP-glucuronosyltransferase activity (aglycone = 4-methylumbelliferone) was unchanged in this experiment.

Measurement of UDP-glucuronosyltransferase activity in skin pieces required addition of UDPGA to the incubation mixtures. No activity occurred when glucose was substituted for UDPGA.

Both interperitoneal and topical treatment of mice with chlordane or its persistent metabolite, oxychlordane, at doses which greatly increased hepatic 7-EC activity, inhibited 7-EC activity in skin pieces. 7-EC activity was inhibited in mouse skin treated *in vitro* with chlordane or oxychlordane. 7-EC activity in mouse hepatic microsomes was inhibited by *in vitro* addition of chlordane but not by *in vitro* addition of oxychlordane.

Chlorinated hydrocarbon and UV radiation effects on xenobiotic-metabolizing enzyme activity will be investigated further. A technique to measure metabolism of benzo(a)pyrene by hairless mouse skin pieces will be developed. Effects of steroids, bacteriostats, such as the halogenated salicylanilides, organic solvents, etc., on xenobiotic-metabolizing enzyme activity in skin will also be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Persistent environmental pollutants, specifically chlorinated organic compounds, are often accumulated in skin. Many other biologically active chemicals are applied to the skin in medicaments, cosmetics, cleaning compounds, etc. Xenobiotic-metabolizing enzymes in the skin may have a role in the locally or systemically expressed toxicity of these compounds. Increased understanding of that role may lead to development of better systems to assess toxicity of chemicals. Manipulation of xenobiotic metabolism may be used to maximize the beneficial effects of chemicals applied to the skin while minimizing toxic reactions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80004-05 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of Factors Affecting Biotransformation of Xenobiotics and Intestinal Transport of Nutrients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. S. Chhabra	Senior Staff Fellow	LP NIEHS
Other:	C. L. Miranda	Visiting Fellow	LP NIEHS
	L. M. Ball	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)

Biometry Branch; Environmental Biology and Chemistry Branch

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this project is to study the effects of environmental pollutants, such as heavy metals, pesticides, and carcinogenic polycyclic hydrocarbons, on the extrahepatic biotransformation of foreign chemicals and intestinal transport of nutrients. The results from this study show that alteration of xenobiotic-metabolizing enzymes in small intestine elicited by exposure to environmental contaminants depends on the type of substrate and animal species used. The intestinal transport of nutrients is generally inhibited in rats exposed to either TCDD or lead salts.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal fractions were isolated by differential centrifugation. Spectrophotometric and spectrofluorometric techniques were used for assay of xenobiotic-metabolizing enzyme activities. Standard liquid scintillation counting and surgical techniques were used for *in vitro* and *in situ* studies of intestinal transport of nutrients.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The characterization of xenobiotic enzymes in small intestine and their comparison with hepatic systems were continued. To determine if there were differences in the inducibility between intestinal and hepatic microsomal drug-metabolizing enzymes: Phenobarbital (PB) or 3-methylcholanthrene (3-MC) was given po or ip to mouse, rat, guinea pig, and rabbit. The microsomal xenobiotic-metabolizing enzymes (ME) studied were ethylmorphine demethylase (EMD), aniline hydroxylase (ANH), aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin deethylase (ECD), and cytochrome P-450 content. Differences in the inducibility of EMD, AHH, and ECD due to the route of administration of PB were observed in guinea pi- mouse, and rat small intestine. There was no difference between PB, po, and PB, ip, in the induction of hepatic DME, but differential effects of po- and ip-administered 3-MC on hepatic AHH and cytochrome P-450 were noted in rat and guinea pig. The induction of intestinal ME varied with the animal species and the substrate used. None of the rabbit intestinal ME were induced by PB or 3-MC treatment, even in rabbits fed semi-purified diet. However, rabbit intestinal ME were not totally resistant to environmental insult since starvation significantly reduced these activities. All hepatic ME studied were induced by PB except AHH in rat. 3-MC induced hepatic ECD in mouse and rat but inhibited it in rabbit. These results suggest that there are quantitative and qualitative differences in induction of ME between small intestine and liver. The inducibility of intestinal ME activities by 3-MC or PB depended on the type of substrate tested and the animal species used.

2) The intestinal transport of glucose and leucine, using both *in vitro* and *in situ* preparations, was studied in rats exposed to lead or TCDD. Adult male rats absorbed glucose more slowly (compared to controls) in *in situ* and *in vitro* after drinking 0.02% w/v lead acetate in their water for 11 weeks. By the 35th week of treatment there were no differences in intestinal absorption of glucose between control and test groups. Adult females had lower levels of glucose absorption *in vivo* than males, but there was no difference due to the lead intake for 13 weeks. Young rats of either sex exposed to lead for one week *in vitro* and later through their own drinking water showed initial slight impairment in both glucose and leucine absorption, which recovered to control levels by 12 weeks of age. Acute exposure of adult male rats to different lead salts (acetate, carbonate, citrate, monoxide, nitrate, and triphenyllead acetate; 65 mg/kg po for three successive days) increased their urinary excretion of 5-aminolaevulinic acid. The intestinal transport of leucine and glucose was affected only by triphenyllead acetate or lead monoxide treatments.

Treatment of adult male rats with TCDD at toxic levels (100 μg/kg po) reduced intestinal glucose uptake in the first few hours following administration of the TCDD. Absorption increased above control levels between one and two weeks after treatment, then declined again after three weeks. Untreated animals, pair-fed to match the decreased food intake seen in treated rats, showed even greater stimulation of glucose uptake after one week. Leucine absorption was depressed throughout.

The role of macro-nutrients (protein, carbohydrate, lipids) and micro-nutrients (minerals, vitamins) on the extrahepatic toxication-detoxication of xenobiotics will continue to be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species and strain differences in the metabolism and response to pollutants by the xenobiotic-metabolizing system may explain both species and strain specific toxicities of pollutants and interaction of pollutants with drugs and chemicals. Pollutants in food and water, as well as in air contact, intestine, and intestinal enzymes, may be a first line of defense of the body against such material.

PUBLICATIONS

Chhabra, R. S. and Fouts, J. R.: Biochemical properties of some microsomal xenobiotic-metabolizing enzymes in rabbit small intestine. Drug Metab. Disp. 4: 208-214, 1976.

Tredger, J. M. and Chhabra, R. S.: Preservation of various microsomal drug-metabolizing components in tissue preparations from the livers, lungs, and small intestines of rodents. Drug Metab. Disp. 4: 451-459, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80005-05 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

In Vitro Metabolism of Xenobiotics by Selected Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Bend	Visiting Scientist	LP NIEHS
Other:	M. O. James	Visiting Associate	LP NIEHS
	T. H. Elmamlouk	Visiting Associate	LP NIEHS
	R. P. Weatherby	Visiting Fellow	LP NIEHS
	R. M. Philpot	Research Chemist	LP NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

We are investigating the biotransformation of foreign organic compounds in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Both cytochrome P-450-dependent microsomal mixed-function oxidases and alkene- and arene oxide-metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control fish and in fish pre-exposed to environmental contaminants such as polycyclic aromatic hydrocarbons or dioxins.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: 1) To compare hepatic and extrahepatic xenobiotic oxidative metabolism, cytochrome P-450 content, and epoxide-metabolizing enzyme activities in several vertebrate and invertebrate marine species of Maine and Florida; 2) to characterize the hepatic mixed-function oxidase (MFO) and epoxide-metabolizing enzyme systems of representative species from Maine and Florida, including the solubilization, purification, and reconstitution of the cytochrome P-450-dependent MFO system; 3) to understand the inability of microsomes prepared from crustacea hepatopancreas to oxidize xenobiotic substrates at significant rates in the presence of NADPH as cofactor; 4) to investigate some xenobiotic conjugating enzymes in marine vertebrates and invertebrates; and 5) to study the effects of environmental contaminant administration on *in vitro* mixed-function oxidation, epoxide hydration, and conjugation reactions by tissues from marine teleosts and elasmobranch.

METHODS EMPLOYED: Subcellular fractions are prepared from tissue homogenates of freshly collected marine specimens. Standard procedures are utilized for the determination of enzyme activities, quantitation of cytochromes, and the solubilization and purification of components of the microsomal mixed-function oxidase system including epoxide hydrase.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Hepatopancreatic microsomes from the spiny lobster, *Panulirus argus*, had high levels of cytochrome P-450 (compared to fish) but had very low or undetectable MFO activities with all substrates tested. However, when cumene hydroperoxide or sodium periodate replaced NADPH in the incubation mixtures, significant MFO activities were present in microsomes prepared from lobster hepatopancreas. Microsomes or cytosol fractions prepared from hepatopancreas of the spiny lobster reduced the *in vitro* NADPH-dependent MFO activities of hepatic microsomes from sheepshead or stingray, whereas boiled hepatopancreas preparations did not affect fish MFO activity. This effect may be due to the actions of digestive enzymes in the hepatopancreas preparations and may explain the lack of NADPH-dependent MFO activity in hepatopancreas microsomes.

2) Parenteral (I.P.) administration of 3-methylcholanthrene (3-MC) to sheepshead (*Archosargus probatocephalus*) caused a 5- to 10-fold increase in hepatic microsomal benzo[a]pyrene hydroxylase (AHH) activity, a 2- to 3-fold increase in 7-ethoxycoumarin O-deethylase (7-ECD) activity, and an approximate doubling of cytochrome P-450 content. 3-MC pretreatment did not affect benzphetamine N-demethylase, epoxide hydrase, or glutathione S-transferase activities. The inductive effect on AHH and 7-ECD activities persisted for six weeks, the longest time point studied so far, after a single 20 mg/kg dose, and was elicited by 3-MC doses as low as 1 mg/kg. Multiple 3-MC doses to stingrays (*Dasyatis sabina*) did not alter hepatic MFO activities, cytochrome P-450 content, or epoxide-metabolizing enzyme activities.

3) A radiochemical assay was developed to measure in vitro activity of taurine N-acyltransferase, using phenylacetyl-coenzyme A as the acyl donor, by quantitating the product phenylacetyltaurine. Activity has been found in hepatic and renal preparations from sheepshead.

4) 1,2,3,4-Dibenzanthracene (DBA) pretreatment (10 mg/kg daily on days 1, 2, and 3; animals sacrificed on day 10) increased AHH activity of hepatic microsomes from little skates (Raja erinacea) up to 30-fold. The apparent V_{max} for hepatic microsomal AHH activity in DBA-treated skates was 20- to 30-fold higher than in control skates, whereas the apparent K_m values were very similar in induced and control fish. The addition of α-naphthoflavone (α-NF) to in vitro incubation mixtures from control skates resulted in increases of AHH activity up to 200% (at 10^{-4} M α-NF). In contrast, α-NF addition to incubation mixtures containing hepatic microsomes from DBA-induced skates resulted in a concentration-dependent inhibition of AHH activity.

5) After digestion of hepatic microsomes from DBA-treated little skates (Raja erinacea) with sodium cholate and fractionation on a DEAE-cellulose column in the presence of Emulgen 911, two cytochrome P-450 peaks were obtained. The first cytochrome peak shows a shift in its absorption maximum to cytochrome P-448 and is eluted from the column with the Emulgen front. The second peak is eluted with a 0-0.5 M KCl gradient and contains cytochrome P-450 with its adsorption maximum at 450-451 nm. After concentration and dialysis the ratio of cytochrome P-448 to cytochrome P-450 varied from 1:1 to 1:1.6 (38-50%). Both types of cytochrome P-450 were essentially free of NADPH-cytochrome c reductase activity and cytochrome b_5.

Two major peaks of epoxide hydrase activity, with styrene oxide or benzo[a]pyrene 4,5-oxide as substrate, were eluted from the same DEAE-columns. The first peak appeared in the void volume and was always contaminated with hemoglobin. The second epoxide hydrase activity peak separated with the cytochrome P-450 and cytochrome P-451 fractions; the cytochrome P-448 fractions were essentially free of epoxide hydrase activity.

6) In general, glutathione S-transferase activities were considerably lower in hepatic tissues of all Maine and Florida marine invertebrate species studied (compared to vertebrates) with benzo[a]pyrene 4,5-oxide, styrene oxide, and octene oxide. In two vertebrate species, the sheepshead and little skate, glutathione S-transferase activity toward the arene oxide substrate, benzo[a]pyrene 4,5-oxide, was higher than activity toward the other substrates, suggesting that these species have altered relative proportions of the several different transferases compared with the other marine vertebrates and mammals investigated, where activity with styrene oxide usually predominated. Glutathione S-transferase activity was widely distributed in extrahepatic tissues of both vertebrate and invertebrate species although hepatic specific and total activities normally were highest.

7) Very high epoxide hydrase activities were found in hepatopancreas of the lobster and spiny lobster (higher than rat), and this enzyme was found to be present in all marine vertebrate and invertebrate animals tested.

In future studies, 1) the effect of controlled exposure of marine fish to different environmental temperatures on hepatic and MFO activities and epoxide-metabolizing enzyme activities will be determined. The response of the hepatic enzyme systems of fish acclimated to various temperatures to xenobiotic administration (3-MC, polybrominated biphenyls, heavy metals, etc.) will also be studied.

2) Taurine N-acyltransferase activity will be characterized with respect to subcellular location and properties of the enzyme and studied in a selection of marine species from Maine and Florida. A suitable species will be chosen for further purification of the enzyme. Properties of purified taurine N-acyltransferase will be compared with the related glycine N-acyltransferase which is present in most mammals, which do not have significant in vivo taurine N-acyltransferase activity towards phenylacetic acid.

3) The two cytochrome fractions (P-448 and P-450) separated from DBA-induced little skates will be further purified and characterized. Differences in substrate specificity (especially with benzo[a]pyrene), position-specific metabolism, substrate binding, and physiochemical properties will be elucidated and compared to these same properties of cytochrome P-450 obtained from hepatic microsomes of untreated little skates.

4) Further purification and characterization of the two epoxide hydrase fractions obtained from DBA-treated skates will also be attempted.

5) Cytochrome P-450 will be solubilized from hepatopancreatic microsomes of lobsters and purified. The cytochrome fraction(s) obtained will be coupled with reductase and lipid fractions obtained from mammals to see if it is catalytically active with a number of xenobiotic and steroid substrates. The fraction(s) will also be tested for its ability to form interaction spectra with various ligands. Microsomal epoxide hydrase will be purified simultaneously. Physiochemical properties of both P-450 and epoxide hydrase will be compared to mammalian and fish enzymes.

6) The number and substrate specificity of glutathione S-transferases from little skate liver will be investigated using ion-exchange chromatographic procedures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products, and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food chain, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and hazard as direct and indirect foodstuffs for man. Comparison of the enzymes involved in metabolizing xenobiotics in different species should lead to a better mechanistic understanding of the overall effects of xeno-

biotics on the animal, and of animals on xenobiotics.

Moreover, a detailed understanding of metabolic activation and deactivation pathways in marine animals may allow us to predict those species most likely to be affected by carcinogens, mutagens, teratogens, or cytotoxins in the environment, and allow us to use them as sentinel or early warning indicators of toxic environmental contaminants.

PUBLICATIONS

Bend, J. R., James, M. O., and Dansette, P. M.: In vitro metabolism of xenobiotics in some marine animals. Ann. N. Y. Acad. Sci. In press.

Bend, J. R., Pohl, R. J., Arinc, E., and Philpot, R. M.: Hepatic microsomal and solubilized mixed-function oxidase systems from the little skate, Raja erinacea, a marine elasmobranch. Proc. Third International Symposium on Microsomes and Drug Oxidations, Berlin, Germany, July, 1976. In Conney, A. H., Estabrook, R. W., Hildebrandt, A. G., and Ullrich, V. (Eds.) Oxford, Pergamon Press. In press.

Philpot, R. M., James, M. O., and Bend, J. R.: Metabolism of benzo[a]pyrene and other xenobiotics by microsomal mixed-function oxidases in marine species. Sources, Effects, and Sinks of Petroleum in the Aquatic Environment. American Institute of Biological Sciences Symposium, Washington, D. C., August, 1976. In press.

James, M. O., Fouts, J. R., and Bend, J. R.: Xenobiotic metabolizing enzymes in marine fish. Pesticides in the Aquatic Environment. XV International Congress of the Entomology Society Symposium, Washington, D. C., August, 1976. In press.

Bend, J. R., Miller, D. S., Kinter, W. B., and Peakall, D. B.: DDE-Induced microsomal mixed-function oxidases in the puffin (Fratercula arctica). Biochem. Pharmacol. 26: 1000-1001, 1977.

Pohl, R. J., Fouts, J. R., and Bend, J. R.: Response of microsomal mixed-function oxidases in the little skate, Raja erinacea, and the winter flounder, Pseudopleuronectes americanus, to pretreatment with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or DBA (1,2,3,4-dibenzanthracene). Bull. Mt. Desert Island Biol. Lab. 15: Report no. 37, 1975.

James, M. O., Pohl, R. J., Peret, D. G., Fouts, J. R., Bend, J. R.: Further studies on epoxide metabolism in vitro by marine species. Bull. Mt. Desert Island Biol. Lab. 15: Report no. 23, 1975.

Bend, J. R., Hall, P., and Foureman, G. L.: Comparison of benzo[a]pyrene hydroxylase (aryl hydrocarbon hydroxylase, AHH) activities in hepatic microsomes from untreated and 1,2,3,4-dibenzanthracene (DBA)-induced male little skates (Raja erinacea). Bull. Mt. Desert Island Biol. Lab. 1976. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80006-06 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Environmental Contaminants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites in Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Bend	Visiting Scientist	LP NIEHS
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LAB/BRANCH

Laboratory of Pharmacology

SECTION

Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The long-range objectives of this project are to study the in vivo uptake, distribution, metabolism, and excretion of single, purified radiolabeled environmental contaminants, such as 2,4,5-trichlorophenoxyacetic acid, polychlorinated biphenyl isomers, and hydrocarbons, in vertebrate and invertebrate marine species that serve as human food sources. The role of environmental temperature and exposure to other pollutants on the processes involved are also investigated. Particular attention is focused upon potential carcinogens, mutagens, teratogens, and cytotoxins that may occur as food residues due to exposure of aquatic animals.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compound, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored, where appropriate. Metabolites are identified by standard procedures.

MAJOR FINDINGS AND PROPOSED COURSE: 1) ^{14}C-2,4,5-Trichlorophenoxyacetic acid was metabolized and excreted rapidly by the dogfish shark, Squalus acanthias, in urine and bile. The major metabolite (> 90% of the excreted ^{14}C) was isolated and identified as 2,4,5-trichlorophenoxyacetyltaurine.

2) ^{14}C-Hexadecane was excreted very slowly from the lobster, Homarus americanus, following parenteral administration into the pericardial sinus (10 mg/kg). After two weeks almost two-thirds of the dosed radioactivity was still in the hepatopancreas, the principal storage site. About 90% of the radioactivity in hepatopancreas from animals sacrificed 7 days after treatment with ^{14}C-hexadecane was unaltered hydrocarbon. However, oxidative metabolites which cochromatographed with hexadecanoic acid and hexadecan-2-ol were also present in this tissue. The slow metabolic turnover of hexadecane by lobsters in vivo is consistent with the very low mixed-function oxidase activities previously found in hepatopancreatic microsomes. ^{14}C-Hexadecane (10 mg/kg) was also eliminated very slowly by the spiny lobster, Panulirus argus, and the hepatopancreas was also the major storage site in this species.

3) Following intravascular administration of ^{14}C-octane (10 mg/kg) to the dogfish shark, very little radioactivity was excreted in the bile or urine (0.6% and 1.6% of dose by day 6 after treatment, respectively). Although there is no doubt that sharks were excreting octane (since the liver contained 80% of the dosed activity 2 hours after treatment but only 16% 6 days after treatment and this was the major storage compartment), the route of removal is not clear. Excretion of the neutral alkane across the gill is a possibility.

4) Flounder skin was found to contain substantial amounts of radioactivity following administration of 2,4,5,2',5'-pentachlorobiphenyl (PCB) (orally or parenterally). Even after frying in oil, the skin of flounder fillets contained significant amounts of radioactivity associated with PCB.

5) The glutathione conjugate of styrene oxide (S-[1-phenyl-2-hydroxyethyl] glutathione-2-^{14}C) was rapidly excreted by winter flounder (70-90% of a 3.9 mg dose in 24 hr urine). The major urinary metabolite, accounting for about 50% of the urinary radioactivity was N-acetyl-S-(1-phenyl-2-hydroxyethyl) glutathione, the corresponding mercapturic acid. Significant amounts of unaltered glutathione derivative (about 30%) were also excreted in urine. The data demonstrates that this teleost has the requisite metabolic pathways for mercapturic acid biosynthesis.

The following aspects of this project will be pursued: 1) The metabolism and excretion of several non-nutritive carboxylic acids will be studied in some Florida vertebrate and invertebrate species for comparison with the data obtained in Maine species. 2) Using xenobiotic conjugates which are excreted in urine and bile, such as ^{14}C-phenacetylglycine, the effect of environmental temperature on the rate of urinary and biliary excretion will be studied in Florida fish that have been acclimated to a variety of temperatures in the laboratory for a prolonged period (2 weeks-1 month) of time. 3) Studies will be continued on the distribution, metabolism, and elimination of selected, radiolabeled aliphatic and aromatic constituents of crude oil, using at least one vertebrate and one invertebrate species from Maine and Florida. The effect of pre-exposure of experimental animals to foreign organic chemicals, such as PCBs, chlorinated insecticides and hydrocarbons, and to heavy metals, on the subsequent uptake, metabolism, and elimination of these aliphatic (octane, hexadecane) and aromatic (benzo[a]pyrene) radiolabeled crude oil constituents will also be studied in Maine and Florida marine animals. 4) Pharmacokinetic parameters for a polychlorinated biphenyl isomer with a low degree of chlorination (probably 4-dichlorobiphenyl) will be investigated in winter flounder that are pretreated with mercury for comparison to identical studies in control fish. 5) The in vivo metabolism and excretion of ^{14}C-styrene oxide, the metabolic precursor of S-(1-phenyl-2-hydroxyethyl)glutathione, will be studied in the winter flounder. Particular emphasis will focus on the relative importance of epoxide hydrase and glutathione S-transferases in the transformation of this toxic alkene oxide.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Marine pollution by chemicals such as crude and refined petroleum products, pesticides, polychlorinated biphenyls, polybrominated biphenyls, and polycyclic aromatic hydrocarbons is a problem of significant importance to human health since many aquatic organisms concentrate such pollutants and man relies upon these species for substantial quantities of food. Carefully controlled pharmacokinetic experiments will identify species that can be sampled in the field to monitor both short and longer term pollutant levels. The study of interaction between environmental contaminants and other xenobiotics, including drugs, food additives, and pesticides, is important since mixtures of such pollutants are always encountered in the environment. Such investigations in marine species, with regard to competition for metabolic and excretion pathways or storage sites, may assist in understanding increased (or decreased) toxicity of pollutant-drug mixtures as they affect higher animals, including man.

PUBLICATIONS

Bend, J. R., Hart, L. G., Guarino, A. M., Rall, D. P., and Fouts, J. R.: Distribution and excretion of [^{14}C]-2,4,5,2',5-pentachlorobiphenyl in the lobster (Homarus americanus) and the dogfish shark (Squalus acanthias). National Conference on Polychlorinated Biphenyls, Chicago, Illinois. Washington, D. C., Office of Toxic Substances, Environmental Protection Agency, 1976, pp. 292-301.

James, M. O. and Bend, J. R.: Taurine conjugation of 2,4-dichlorophenoxyacetic acid and phenylacetic as a major metabolic pathway in two marine species. Xenobiotica 6: 393-398, 1976.

Foureman, G. L., Hall, P., and Bend, J. R.: Distribution of 1-^{14}C-hexadecane in the lobster, Homarus americanus, at various times after injection into the pericardial sinus. Mt. Desert Island Biol. Lab. Bull., 1976. In press.

Guarino, A. M., Rieck, G., Arnold, S., Fenstermacher, P., Bend, J. R., Knutson, M. J., and Anderson, J. B.: Distribution and toxicity of selected water pollutants in the dogfish shark, Squalus acanthias. Mt. Desert Island Biol. Lab. Bull., 1976. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80007-06 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER XXX

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Several arene oxides and alkene oxides are known to react covalently with macromolecules, including nucleic acids, and to transform cells in vitro, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the mixed-function oxidases, which convert unsaturated hydrocarbons to epoxides, and the further metabolism of arene and alkene oxides by soluble fraction glutathione S-transferases and microsomal epoxide hydrase in hepatic and extrahepatic tissues (including testis, ovary, and adrenal). The relative quantitative importance of the two pathways for epoxide metabolism is being studied in vitro and in isolated perfused rat liver and rabbit lung preparations, which have intact cellular structure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: 1) The characterization of the glutathione S-transferases of rabbit lung as compared to the hepatic enzymes; 2) to compare the biotransformation of chemically reactive epoxides and their unsaturated hydrocarbon metabolic precursors in vitro and in isolated perfused liver and lung preparations; 3) to correlate dosage levels with saturation or alteration of epoxide-metabolizing pathways and toxicity (including histopathology and covalent binding of radioactivity to macromolecules) in isolated perfused organs; 4) to study epoxide metabolism in germinal and steroidogenic tissues; and 5) to synthesize model substrates to assess mechanistic relationships between detoxication pathways and chemical-mediated toxicity (mutagenesis, carcinogenesis).

METHODS EMPLOYED: Differential centrifugation, spectroscopic, fluorometric and radiochemical assays, organ perfusion procedures, ion exchange, thin-layer, paper, and high pressure liquid chromatography, and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Rabbit liver 176,000xg supernatant fraction was shown to have at least six separable peaks of glutathione S-transferase activity as eluted from carboxymethylcellulose (CMC) columns. The six activity peaks were detectable with both 1-chloro-3,4-dinitrobenzene (DNCB) and benzo[a]pyrene-4,5-oxide (BPO) as substrates, whereas styrene oxide (SO) conjugating activity was localized in only one activity peak. SDS gel electrophoresis of the six activity fractions demonstrated that only one protein band (MW 24,000) was common to all six fractions. Significant variability of individual activity peaks in the DNCB substrate profiles could be demonstrated in single liver experiments.

2) Rabbit lung 176,000xg supernatant fraction was fractionated by procedures similar to those described for the hepatic glutathione S-transferases. Six peaks of transferase activity towards BPO and DNCB were identified. In contrast to the hepatic transferases, SO conjugating activity was present in at least two of the six activity peaks.

3) Isolated perfused rabbit lung metabolized benzo[a]pyrene (BP) at an overall rate of 5.9 ± 1.8 nmole/min/g lung, which is similar to rates found with pulmonary microsomes. Phenols, dihydrodiols, and glutathione conjugates were identified as metabolites. Intermediate arene oxides were nonoxidatively metabolized to dihydrodiols much more rapidly than to glutathione conjugates (5- to 10-fold). Pretreatment of the rabbits with 3-methylcholanthrene slightly, but significantly, increased the rate of BP metabolism (1.6-fold) in perfused lung.

4) Benzo[a]pyrene 4,5-oxide (BPO) was metabolized by the isolated perfused lung preparation primarily by nonoxidative pathways (epoxide hydrase and glutathione S-transferases). BPO hydration rates in the organ were almost

equal to those predicted from in vitro studies with pulmonary microsomes, whereas conjugation with glutathione occurred much more slowly in the perfused lung than in 176,000xg supernatant fractions of lung homogenate. BPO bound covalently to lung proteins but not appreciably to DNA.

5) Styrene oxide (SO) metabolism at three different concentrations (50 μmole, 100 μmole, and 500 μmole/liver) was studied in the isolated perfused rat liver. Almost all of the biliary metabolites were thioether conjugates, principally the corresponding glutathione conjugate, S-(2-hydroxy-1-phenyl) ethylglutathione. The amount of radioactivity excreted in bile did not increase proportionately with dose, suggesting that the system transporting conjugates into bile was saturated.

The predominant metabolites in perfusate (after 60 min) were styrene glycol and thioether metabolites, in approximately equal amounts. This demonstrated that epoxide hydrase and glutathione S-transferases were both quantitatively important in the biotransformation of SO over the dosage regimes studied.

Covalent binding of SO-associated radioactivity to protein was examined. There was a positive correlation between radioactivity covalently bound and dose. After perfusion, livers were examined histologically to assess cellular damage. At the lowest dose (50 μmole), there was no lysing of cells but there were slight alterations in the periportal area. At the highest dose, centrilobular damage was much in evidence. At the intermediate dose some livers showed centrilobular toxicity which others did not. Increased SO doses resulted in increased glutathione depletion.

6) Glutathione S-transferase activity of rat serum, with SO, BPO, DNCB, and 1,2-dichloro-4-nitrobenzene as substrates, was less than 1% hepatic activity. Neonatal animals had barely detectable serum enzyme activities which increased gradually with age. Serum transferase activities in male rats were about 60% greater than those in females. Pretreatment with carbon tetrachloride (1 ml/kg for 24 hrs) caused an 11-fold increase in specific serum transferase activities, which was associated with a 40% decrease in hepatic activities. Collectively, the data suggest that serum glutathione S-transferase activities may be due to a leak of soluble enzymes from liver.

7) The perinatal development of glutathione S-transferase activity was followed in rat ovary and adrenal. In adrenals, transferase specific activities were high in 1-day-old rats (about 50% adult hepatic values with SO and BPO) and did not change appreciably with age. Ovaries of 12-day-old rats had approximately 25% adult hepatic values. Activity developed gradually with age and reached a maximum by 56-days-of-age.

8) A 90% increase in specific glutathione S-transferase activity was observed in the ovaries of pregnant rats (relative to virgin female animals) with SO. Similarly, specific cytochrome P-450 and cytochrome b_5 contents of microsomes prepared from rat ovaries during mid- or late-pregnancy were 4- to 5-fold higher than in control animals. No concomitant changes occurred in liver or

ovarian mitochrodrial cytochrome P-450 or b_5 content. Microsomal aryl hydrocarbon hydroxylase and epoxide hydrase activities were not increased in ovaries of pregnant rats.

9) The isomeric cholestane-3-ol-5,6-epoxides were synthesized by unequivocal routes and with considerable improvement over previous work.

The proposed course of this project includes:

1) The number and substrate specificity of glutathione S-transferases from rat ovary will be investigated using ion exchange chromatographic procedures. The previously demonstrated increase in BPO and SO conjugating activities of pregnant vs. nonpregnant animals will be investigated to determine if this is due to an induction of all transferases or induction of specific ovarian transferases.

2) The number and substrate specificity of rabbit renal glutathione S-transferases will be investigated. Binding of organic anions to the partially purified enzyme preparations will be compared with renal tissue slice experiments. A comparison of organic anion binding to the partially purified hepatic and renal transferases will be attempted.

3) The effect of administering prolactin, progesterone, and hydrocortisone to rats on ovarian epoxide-metabolizing enzyme activities will be determined in attempts to identify the factors responsible for increased ovarian glutathione S-transferase activity during pregnancy.

4) The metabolism of benzo[a]pyrene 7,8-dihydrodiol will be investigated in the isolated perfused rabbit lung and rat liver preparations. Covalent binding to RNA, DNA, and protein will be quantitated in each case and major metabolites will be quantitated and identified.

5) The metabolism of styrene oxide will be studied in isolated perfused livers from rats pretreated to alter glutathione S-transferase and/or epoxide hydrase activities. Histological damage, metabolic profile, and covalent binding of SO-associated radioactivity will be related to SO dose and will be compared to similar data from control animals.

6) The metabolism of styrene will be studied in isolated perfused liver and lung preparations. The quantitative distribution of epoxide hydrase-derived metabolites vs. glutathione S-transferase-derived metabolites will be compared with earlier studies using styrene oxide as substrate.

7) The mutagenesis of the isomeric cholestane-3-ol-5,6-epoxides will be tested in bacterial and yeast strains. The compounds will also be synthesized in a radioactive form to test their substrate specificity for glutathione S-transferases.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The conjugation of xenobiotics or their metabolites which are chemically reactive (e.g., alkene and arene oxides) by liver, lung, gut, and other extrahepatic tissues may play a significant role in the overall toxicity of agents which enter the body via the pulmonary system or the alimentary canal. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants and may also be related to chemical carcinogenesis and teratogenesis. In the real-life situation one is very rarely exposed to a single xenobiotic; rather, one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, hydrocarbons, etc. For this reason investigations concerning interactions between more than one foreign compound are of relevance to environmental health.

PUBLICATIONS

Ryan, A. J., James, M. O., Ben-Zvi, Z., Law, F. C. P., and Bend, J. R.: Hepatic and extrahepatic metabolism of 8-^{14}C-styrene oxide. Environ. Hlth. Persp. 17: 135-144, 1976.

Harper, C., James, M. O., Devereux, T. R., Patel, J. M., Bend, J. R., and Fouts, J. R.: Characteristics and development of drug metabolism by pulmonary microsomes. Agents and Actions 6: 527-530, 1976.

James, M. O., Foureman, G. L., Law, F. C. P., and Bend, J. R.: Perinatal development of epoxide hydrase and glutathione S-transferase in hepatic and extrahepatic tissues of the rabbit and guinea pig. Drug Metab. Disp. 5: 19-28, 1977.

Bend, J. R., and Hook, G. E. R.: Hepatic and Extrahepatic Mixed-function Oxidases. In Lee, D. H. K. (Ed.): Handbook of Physiology, Section 9: Reactions to Environmental Agents. Bethesda, Md., American Physiological Society, 1977, pp. 419-440.

Jerina, D. M. and Bend, J. R.: Glutathione S-transferases. In Jollow, D., Kocsis, J. J., Snyder, R., and Vainio, H. (Eds.): Biological Reactive Metabolites: Formation, Toxicity and Inactivation. New York, Plenum Press, 1977, pp. 207-230.

Ryan, A. J. and Bend, J. R.: Metabolism of styrene oxide in the isolated perfused rat liver: Identification and quantitation of major metabolites. Drug Metab. Disp. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80008-03 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Transport and Metabolism of Prostaglandins [PGs] in Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Pharmacokinetics Section	LP NIEHS
	M. W. Anderson	Mathematician	BB NIEHS
Other:	A. G. E. Wilson	Visiting Associate	LP NIEHS
	A. Chaudhari	Visiting Fellow	LP NIEHS
	D. Crutchley	Visiting Fellow	LP NIEHS
	H. J. Hawkins	Bio. Lab. Tech	LP NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology and Chemistry Branch

LAB/BRANCH
Laboratory of Pharmacology; Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.45	1.0	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the long-range goal of this project to study the inactivation of prostaglandins by the lungs and other tissues. The effects of environmental agents on the pulmonary transport and metabolism of prostaglandins is being investigated, and these effects are being related to pulmonary toxicity and damage caused by the environmental agents. Topics of current interest are the structural requirements of a prostaglandin needed for transport to intracellular spaces, the effect of oxidant gases on transport, and the potentiation of inflammatory reaction by inhibition of pulmonary inactivation of prostaglandins.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: An isolated perfused rat, guinea pig, or rabbit lung was used to examine the uptake, metabolism, and efflux of prostaglandins (PGs) and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography techniques. The unidirectional flux of PG into the lung was measured by extrapolation of the net uptake velocity of PG to zero time.

MAJOR FINDINGS AND PROPOSED COURSE: PGs are accumulated into lung tissue by a carrier-mediated process and are subsequently degraded by pulmonary enzymes to either 15-keto PG or 15-keto-13,14-dihydro PG. The uptake, metabolism, and subsequent release of parent PG and metabolites are remarkably efficient and rapid.

Structural activity studies were done to determine the critical moities of the PG essential for transport into lung tissue. The following PGs were substrates for the pulmonary transport system: PGE_1, PGE_2, $PGF_{1\alpha}$, $PGE_{2\alpha}$, PGD_2, 15(S) methyl-$PGF_{2\alpha}$, and $PGF_{2\alpha}$. PGA_1, PGB_1, 15-keto $PGF_{2\alpha}$, 15-(R) methyl $PGF_{2\alpha}$, 15-keto PGE_1, 15-keto 13,14-dihydro PGE_1, 13,14-dihydro PGE_1, 13,14-dihydro $PGF_{2\alpha}$, the methyl esters of $PGF_{1\alpha}$, PGE_1, $PGF_{2\alpha}$, and PGE_2, 15 epi-PGE_2 and 15 epi-$PGF_{2\alpha}$ were either poor substrates or not substrates for the pulmonary transport system. From these studies we have concluded that there are three critical portions of the PG molecules necessary for transport into lung tissue:
(1) acid group at C-1
(2) oxygen function, particularly a hydroxyl group at C-1
(3) a hydroxyl group at C-15 in the S configuration.

Small changes in the geometric relationship of these three portions can greatly alter the lung's affinity to transport and metabolize a PG. For example, conversion of the 15 hydroxyl from an S to R configuration greatly reduces transport. Although we could not elucidate what is responsible for the substrate specificity, it is possible that the critical regions are necessary for the binding of a PG to a carrier molecule.

We have also investigated the effect of various chemicals on the transport system. The PG antagonist SC-19222 and 7-oxo-prostanoic acid did not inhibit removal while PPP and DPP were potent inhibitors. PPP and DPP possess a large number of hydroxyl groups, with the distance between hydroxyls essentially the same as the distance between the oxygen function on PG molecules at C-9 and C-15. This may account for DPP and PPP's inhibitory activity.

It has been previously demonstrated that pulmonary inactivation of PG is highly species dependent. For example, PGA_1 is not metabolized by rat but is metabolized by guinea pig, whereas PGE_1 is metabolized by both species. We have studied the uptake and metabolism of PG in rats, rabbits, and guinea pigs using the isolated perfused lung and have found little or no species difference in either transport or metabolism. PGE_1 but not PGA_1 is degraded by all species.

However, if the conditions of perfusion were altered and albumin was removed from the perfusate, then both PGA_1 and PGE_1 would be degraded by the IPL. Thus, the presence of albumin in the perfusate can greatly alter the pulmonary transport and metabolism of PGA_1. Previously reported studies which showed PGA_1 metabolism did not have albumin in the perfusate, whereas studies which reported no pulmonary degradation of PGA used albumin in the perfusate. Thus, little or no significant species dependence exists for the pulmonary inactivation of PG.

We have also investigated the metabolism of PG by glutathione-S-aryl transferase. Previous studies have shown that PGA_1 is metabolized to water-soluble glutathione conjugates. We have shown that the 15-keto $PGF_{2\alpha}$ is also converted to glutathione conjugates by the enzyme glutathione-S-aryl transferase. Activity is highest in liver followed by kidney and lung. The presence of the 13-14 double bond is essential for conjugation and indicates that an α-β-unsaturated keto is the critical portion of the PG molecule required for conjugation. Since many 15-keto PGs have biological activity, conjugation by glutathione may be a new and important detoxification system for PG.

We have studied the effect of exposure of rats and guinea pigs to 100% O_2 on the pulmonary inactivation system. Exposure to 100% O_2 significantly depresses the enzyme system, prostaglandin dehydrogenase (PGDH), without effect on the transport system. NAD^+ and $NADP^+$ PGDH succinic dehydrogenase, but not glucose-6-PO_4 dehydrogenase, was inhibited by exposure to O_2. Kinetic studies indicate an uncompetitive inhibition of PGDH on exposure to O_2 which is consistent with the hypothesis that inhibitor was generated by the exposure. Further studies are in progress to further substantiate this hypothesis.

The proposed course is as follows: 1) To further study the effect of anti-inflammatory drugs on PG transport in the lung; 2) to examine the effect of exposure of rats to airborne pollutants on PG detoxification system; 3) to use isolated cell systems to examine the mechanisms for the transport of PG into and out of cells and apply the information obtained from this study to the intact lung system; 4) to examine the effect of exposure of animals to pollutants on the inflammatory release of vaso-active chemicals from the lung; and 5) to further examine the formation of glutathione conjugates of 15-keto PG.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes. The lung is an important site for the synthesis and metabolism of PGs; alterations in the PG transport and metabolism systems may be related to toxic effects of exposures to pollutants and induction of lung diseases.

PUBLICATIONS

Anderson, M. W. and Eling, T. E.: Prostaglandin removal and metabolism by isolated perfused rat lung. Prostaglandins 11: 645-677, 1976.

Eling, T. E. and Anderson, M. W.: Studies on the biosynthesis, metabolism, and transport of prostaglandins by the lung. Agents and Actions 6: 543-546, 1976.

Eling, T. E., Hawkins, H. J., and Anderson, M. W.: Structural requirements for and the effect of chemicals on the pulmonary inactivation of prostaglandins. Prostaglandins. In press.

Hawkins, H. J., Wilson, A. G. E., Anderson, M. W., and Eling, T. E.: Uptake and metabolism of prostaglandins by isolated perfused lung: Species comparison and the role of plasma protein binding. Prostaglandins. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80009-06 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Functions of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. G. E. Wilson	Visiting Associate	LP NIEHS
Other:	T. E. Eling	Head, Pharmacokinetics Sec.	LP NIEHS
	M. W. Anderson	Mathematician	BB NIEHS
	A. Kung	Visiting Fellow	BB NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology and Chemistry Branch

LAB/BRANCH

Laboratory of Pharmacology; Biometry Branch

SECTION

Pharmacokinetics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	1.6	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The goal of this project is to examine pharmacokinetic functions of the lung with regard to both endogenous and exogenous chemicals. The pharmacokinetics of these chemicals will be related to environmentally induced pulmonary damage. An isolated perfused lung is used which permits examination of the pharmacokinetics of chemicals reaching the lungs both from the circulatory system and from the inhaled air.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating blood supply, and an air supply. The lungs are ventilated with 95% air-5% CO_2 by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a blood reservoir directly above the lungs to which the labeled chemicals were added and from which samples of blood were removed for analysis. The apparatus was modified so that the rates of uptake and the rates of efflux of chemicals from lung could be examined.

Metabolites of the compounds as found in the blood and lung were studied by thin-layer, paper, and gas liquid chromatographic techniques. Viability of the perfused lung was confirmed by physiologic parameters as well as by light and electron microscopy.

The perfused lung is designed to allow examination of the metabolism and retention of inhaled chemicals. The trachea is connected directly to an aerosol generator and the appearance of the chemical and its metabolites in the circulating media is monitored.

MAJOR FINDINGS AND PROPOSED COURSE: We have studied the in vivo disposition of imipramine (IMIP) in rabbits. The noneffluxable pool observed in the isolated pefused lung (IPL) appears to be a pool with a half-life in excess of 6 hr. The metabolite of IMIP, desimipramine, also appeared to form this persistence pool. The major metabolite in the rabbit 12-24 hr after the administration of IMIP was water-soluble conjugates.

Considerable effort was devoted to further developing the isolated perfused lung to study the uptake, distribution, and metabolism of inhaled chemicals. The problems of proper inhalation techniques for IPL and safety consideration were apparently solved. Major efforts will be devoted to investigating the pharmacokinetics of inhaled chemicals.

The proposed course is as follows: 1) To further develop the IPL to study the pharmacokinetics of inhaled chemicals; 2) to examine the uptake and metabolism of environmental agents or model chemicals and relate the induction of pulmonary damage; and 3) to elucidate the mechanism(s) and differences in the pulmonary metabolism of inhaled and blood-borne chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a method for the perfusion of an isolated intact lung allows studies with the lung uncomplicated by other organ functions. This preparation is perfused and ventilated similarly to an intact organ and thus good correlation of in vivo and in vitro studies is likely.

The lung can act as an important site for accumulation of xenobiotics and this accumulation could result in detoxification or altered toxicity.

In addition, those xenobiotics which accumulate in the lung could affect the accumulation and storage of other exogenous compounds or of endogenous substances such as norepinephrine, prostaglandins, and 5-hydroxytryptamine. The elucidation of the mechanism of accumulation could afford a means for predicting possible toxic effects and interactions of one chemical with another.

PUBLICATIONS

Anderson, M. W., Eling, T. E., and Philpot, R. M.: Metabolic Functions of Lung. In Lenfant, C. (Ed.): Monograph 5 of the series entitled "Lung Biology in Health and Diseases." Uptake, Disposition, and Metabolism of Chemicals by the Lung. New York, Marcel Dekker. In press.

Wilson, A. G. E., Law, F. C. P., Eling, T. E., and Anderson, M. W.: Uptake, metabolism, and efflux of methadone in "single pass" isolated perfused rabbit lungs. J. Pharmacol. Exp. Ther. 199: 360-367, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80010-06 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Prostaglandin and Thromboxane Biosynthesis and Release by Lungs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Pharmacokinetics Section	LP NIEHS
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Other:	A. Chaudhari	Visiting Fellow	LP NIEHS
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	O. Hernandez	Visiting Associate	EBCB NIEHS
	H. J. Hawkins	Bio. Lab. Tech.	LP NIEHS
	W. Lynn	Professor, Dept. of Medicine	Duke University

COOPERATING UNITS (if any)
Inhalation Toxicology Section, Environmental Biology and Chemistry Branch; Duke University

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Laboratory of Pharmacology; Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The goal of this project is to study the biosynthesis, release, and metabolism of prostaglandins (PGs) and thromboxanes (TX) by pulmonary tissue and to relate altered PG biosynthesis, release, or metabolism to pulmonary diseases. Also of interest is to study PGs as early indicators of pulmonary damage induced by exposure to environmental agents. Emphasis is being placed on studying the biochemical mechanisms that control the release of PG precursors, unsaturated fatty acids, from lung phospholipids and, thus, partially controlling PG biosynthesis. Isolated perfused lung and tracheal ring cultures will be used in these studies. Detoxification of TX and the biological activity of TXA_2 and PG will be studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandins (PGs) are analyzed by a combination of quantitative thin-layer chromatography and liquid scintillation counting. The radioactive PGs produced by lung tissue from the precursor ^{14}C-arachidonic acid are extracted from the incubation medium and separated on thin-layer plates. The radioactive areas are measured by liquid scintillation techniques. Guinea pig or rat lung serves as an enzyme and co-factor source for both biosynthesis and metabolism. Further identification of PG uses a combination of thin-layer chromatography, continuous gradient elution column chromatography, and mass spectrometry. PG metabolism was estimated by incubation of ^{3}H-$PGF_{2\alpha}$ with the enzyme source, extraction from the incubation mixtures, and separation of nonmetabolized ^{3}H-$PGF_{2\alpha}$ from metabolites. Metabolism was measured by the disappearance of ^{3}H-$PGF_{2\alpha}$. The isolated perfused lung (IPL) is used to study release of PG from the lung as a result of environmental insults.

MAJOR FINDINGS AND PROPOSED COURSE: The lung has been found to produce, from arachidonic acid, PGE_2, $PGF_{2\alpha}$, PGX, thromboxane A_2 and B_2 (TXA_2 and TXB_2), 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT). We have found that guinea pig lung produces greater amounts of TX and PGX from PGE_2 and $PGF_{2\alpha}$. This is of importance since TX and PGX have been shown to be approximately 1000 times more biologically active than PGE_2 or $PGF_{2\alpha}$. In addition, guinea pig lung does produce some HETE which has been shown to be chemotactic. We have found that rat lung produces small amounts of PG and PG-like substances and equal amounts of TXB_2, $PGF_{2\alpha}$, and PGE_2. No information is available on PG synthesis in other species and in man. Cells obtained in alveolar washing (PMN, macrophages, etc.) also synthesize PG but the kinds and amount of the PG have not been examined.

The release of PG and TX from lung tissue can be measured using bioassays and radioactivity techniques. Bioassays for PG and TX were developed in this laboratory based on previously published techniques. To use radiolabeled methods, it was necessary to label the arachidonic acid (AA) pool in pulmonary tissue. ^{14}C-AA was rapidly removed from the circulation and stored in phospholipids. On stimulation of lung by standard techniques, PG and TX were released but the amount of radioactivity release was small in comparison to the total amount of ^{14}C-AA incorporated into lung tissue. Current studies are in progress to increase the specific activity of labeled AA esterfied with phospholipid. Additional methods for measuring PG and TX are also under consideration, for example, radioimmunoassays and the use of C-13-labeled AA.

In addition to studies using the isolated perfused lung, we are currently developing tracheal ring culture methods. These cultures can be used to study the incorporation of ^{14}C-AA into lung tissue or mechanism(s) for the release of AA and its subsequent conversion to PG and TX. Tissue cultures offer many advantages in the IPL for studying the mechanism of release.

Little is known about the mechanism(s) of biological action of TXA_2. Due to its instability and unusual structure, TXA_2 has not been chemically synthetized. We have developed a model synthesis for TXB_2 and therefore TXA_2. A direct synthesis of TXB_2 has been accomplished. The synthetic sequence leading to TXB_2 starts with the commercially available α-methylglucoside and leads in seven steps to an intermediate from which TXB_2 may be produced by standard synthetic operations. The advantages inherent to this synthesis may be summarized as follows: a) the material produced is enantiomerically pure and thus the waste involved in working with racemic mixtures is avoided, b) the operations involved do not require specialized equipment and may be scaled up without much loss in efficiency.

Our intention is to synthesize a variety of TXA_2 model compounds and test these chemicals for biological activity. With pure ^{14}C-TXA_2 we will examine the interaction of TXA_2, its mechanism of action, and its role in pulmonary disease.

The proposed course is as follows: 1) Examination of PG synthesis in the lung of rat, rabbit, dog, and monkey. Human lung samples will be obtained from Duke University. 2) Study the release of PG from the lung as a result of environmental insults. Little information is available on the release of PG from the lung into the circulation and alveolar spaces as a result of various stimuli. 3) Measure the levels of PG and PG-like materials in the alveolar washings from human patients with pulmonary disease and relate PG to these diseases. 4) Study PG systems in the cellular components obtained from humans suffering environmentally induced lung diseases. 5) Study the mechanism responsible for the release of PG precursor, arachidonic acid, from lung phospholipid. Examine the various cellular preparations for PG synthetase activity and specific phospholipases. Washings from patients with bronchial asthma will be studied for possible increased or altered phospholipase activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1) PG and PG-like substances represent a class of endogenous substances which possess unique biological activity. PGs have been implicated in inflammation and control of smooth muscle tone. The isolation and purification of the cyclic intermediates and the thromboxanes opened a whole new vista of biological effects related to the PGs. Cyclic intermediates and thromboxanes have very potent effects on smooth muscle, lung airways, platelets, and adenyl cyclase. Because PG and PG-like substances are not stored in tissues, control of biosynthesis (release of arachidonic acid and conversion to PG and PG-like substances) and metabolism is most important in understanding their physiological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80014-04 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism of Polychlorinated Biphenyls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	LP NIEHS
	J. R. Hass	Staff Fellow	EBCB NIEHS
	S. Kato	Visiting Fellow	EBCB NIEHS
	J. D. McKinney	Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

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SECTION
Pharmacokinetics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Metabolism to more polar compounds is a prerequisite to polychlorinated biphenyl (PCB) excretion by animals. Two adjacent unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs. The major metabolites of PCBs are mono- and di-hydroxylated derivatives which are usually excreted as glucuronide conjugates. There is good evidence to suggest that the PCBs are metabolized by hepatic mixed-function oxidases to their respective hydroxylated derivatives via arene oxide intermediates.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The studies have utilized ^{14}C-labeled PCBs and in vivo metabolism by the rat to obtain the necessary metabolites. The metabolites were isolated by various extraction techniques, purified by thin-layer and column chromatography, and identified by mass spectral and nuclear magnetic resonance analysis. A second approach which is being utilized in the study of the very slowly metabolized PCBs is the synthesis of the anticipated metabolites. These "synthetic metabolites" are then used to identify the authentic metabolites by cochromatography, comparison mass spectrometry, and isotope dilution.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The major metabolites of the PCBs are mono- and di-hydroxylated PCBs and there is a possibility of an arene oxide intermediate. Metabolism appears to be a prerequisite to PCB excretion. Two adjacent unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs.

2) The major metabolites of 4-chlorobiphenyl have been isolated and identified as: 4-hydroxy-4'-chlorobiphenyl; 3-hydroxy-4'-chlorobiphenyl; 3,4- dihydroxy-4'-chlorobiphenyl, and 3-hydroxy-4-methoxy-4'-chlorobiphenyl. These metabolites account for approximately 60, 7, 23, and 3 percent, respectively, of the radioactivity extracted from the excreta of treated rats.

3) The major metabolite (90%) of 4,4'dichlorobiphenyl has been isolated and identified as 4-hydroxy-3,4'-dichlorobiphenyl. Traces of the 3-hydroxy-4,4'-dichlorobiphenyl have also been isolated.

4) The metabolites of 2,4,5,2',5'-pentachlorobiphenyl have been described and are 3-hydroxy-2,5,2',4',5'-pentachlorobiphenyl, 3,4,-dihydro-3,4-dihydroxy- and 3,4-dihydroxy-pentachlorobiphenyl. Only approximately 13% of this compound is excreted as the parent compound, 60% of the radioactivity excreted was in the form of the monohydroxy, and about 25% was in the form of the dihydrodiol and dihydroxy metabolites.

The proposed course is as follows: The major anticipated metabolites of 3,4,3',4'- and 3,5,3',5'-tetrachloro-biphenyls, 2,4,5,2',4',5'-, 2,4,6,2',4',6'-, 2,3,5,2',3',5'-, and 2,3,6,2',3',6'-hexachlorobiphenyls are currently being synthesized. The metabolism of each of these PCBs is also being studied in the rat. Upon isolation and identification of the metabolites of these PCBs, the toxicology and possibly other biological effects, e.g., estrogenic effects of the metabolites will be the subject of further investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The polychlorinated biphenyls are ubiquitous environmental contaminants, and knowledge of their biotransformation and the identification of their metabolites is essential to our understanding of their importance as potential hazards to man and his environment. We have evidence that arene oxides are

formed in the metabolism of PCBs. Arene oxides have been implicated in a number of undesirable biological reactions including teratogenesis, carcinogenesis, and tissue necrosis.

PUBLICATIONS

Chen, P. R., McKinney, J. D., and Matthews, H. B.: Metabolism of 2,4,5,2'5'-pentachlorobiphenyl in the rat: Qualitative and quantitative aspects. Drug Metab. Disp. 4: 362-367, 1976.

Hass, J. R., Jao, L. T., Wilson, N. K., and Matthews, H. B.: Metabolism of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl in the rat: Qualitative and quantitative aspects. J. Agr. Food Chem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80015-04 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Transport of Xenobiotics by Blood and Its Constituents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	LP NIEHS
	M. W. Anderson	Mathematician	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology; Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The polychlorinated biphenyls (PCBs) and certain chlorinated pesticides are readily taken up by the blood and its components and transported to the liver where they are readily released. Our studies suggest that these compounds are distributed between the red blood cells, albumin, and lipoproteins at ratios which vary according to the lipophilicity of the chlorinated hydrocarbon involved. Thus, the process is thought to be a passive one in which the physical and chemical properties of the chlorinated hydrocarbons determine the blood constituent in which they will be most concentrated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The distribution of ^{14}C-labeled xenobiotics such as biphenyl, eight different polychlorinated biphenyls (PCBs), three chlorinated pesticides, one nonchlorinated pesticide, and an endogenous compound, a steroid hormone, are being studied in various blood fractions. Methods employed included adsorption and molecular seive chromatography, density gradient centrifugation, and electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: We have investigated the nature of the association between PCBs and blood components. Both *in vivo* or *in vitro* PCBs are associated with red blood cells (RBCs), albumin, and lipoproteins. The fraction associated with RBCs is most rapidly removed from the blood by the tissues while the fractions associated with the plasma proteins are released more slowly. Gel filtration of plasma containing a PCB showed that the lipoprotein fraction contained a higher specific activity (dpm/mg of protein) than did albumin. Other methods of plasma fractionation, e.g., chromatography on glass powder columns and gradient centrifugation, have confirmed the affinity of the PCBs for the lipoproteins and have shown that the relative amounts of a given PCB associated with the lipoproteins and the albumin is related to the lipophilicity of the given PCB. The results of these studies imply that the association between PCBs and blood proteins is nonspecific and that the factors controlling the relative amounts of PCB associated with the different classes of proteins are biophysical rather than biochemical.

We have shown no evidence that the transport of lipophilic xenobiotics interferes with the normal transport of endogenous materials.

We think that the project has been investigated in sufficient detail to confirm that the transport of lipophilic xenobiotics by blood is by passive and nonspecific association of these compounds with blood components. Furthermore, under normal circumstances the transport of such xenobiotics by blood will not interfere with the transport of endogenous compounds. Thus, the results of this project are currently being prepared for publication. Upon publication of the results, the project will be terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental exposure of man to a large number of xenobiotics via air, water, or food contamination is significant. These materials are transported by the blood to various detoxication, excretion, or storage sites within the body. Thus, if the transport of any one of these materials should interfere with the normal transport of endogenous materials by the blood then it could result in an imbalance of homeostasis and serve as a mechanism of toxic effect.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-04 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics of Chlorinated Xenobiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	LP NIEHS
	M. W. Anderson	Mathematician	BB NIEHS
Other:	N. M. Morales	Visiting Fellow	LP NIEHS
	D. B. Tuey	Guest Worker (UNC)	BB NIEHS
	R. L. Dedrick	Section Chief	BEIB DRS
	R. Lutz	Chemical Engineer	BEIB DRS

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Pharmacology; Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.9	2.5	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Polychlorinated biphenyls (PCBs), 2,4,5,2',4',5'-hexabromobiphenyl (PBB), and Kepone are readily absorbed from the gut, rapidly removed from the blood, and initially stored in the liver and muscle. At later time points the less chlorinated PCBs are metabolized and excreted while the highly chlorinated PCBs and the PBB are translocated from the liver and muscle to the skin and adipose tissue which are the sites of long-term storage. The degree and rate of metabolism and the ultimate biologic half-life of PCBs is determined by the degree and position of chlorination. The degree of PCB metabolism decreases as the chlorine content increases, and the effect of increasing chlorine content is most marked when the chlorine atoms are arranged so that the biphenyl molecule does not have two adjacent unsubstituted carbon atoms. The PBB and Kepone do not have two adjacent unsubstituted carbon atoms and are not subject to an appreciable amount of metabolism. Kepone is stored primarily in the liver and is excreted in the bile at a rate proportional to the liver concentration. On the other hand, the PBB is stored primarily in the adipose tissue, is subject to very little excretion, and thus has an infinite half-life in the male rat.

PROJECT DESCRIPTION

METHODS EMPLOYED: The distribution, accumulation, and excretion of eight ^{14}C-labeled PCBs (4-chloro-, 4,4'-dichloro-, 3,5,3',5'-tetrachloro-, 3,4,3',4'-tetrachloro-, 2,4,5,2',5'-pentachloro-, 2,3,6,2',3',6'-hexachloro-, 2,4,6,2',4',6'-hexachloro-, 2,4,5,2',4',5'-hexachloro-biphenyl), Kepone, and a polybrominated biphenyl (PBB) (2,4,5,2',4',5'-hexabromobiphenyl) were studied after iv and oral administration to rats. Four of the PCBs (4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachloro-biphenyl) were also studied in the mouse. The major tissues and excreta were sampled at times varying from a few minutes to several weeks posttreatment. Distribution and excretion were studied under conditions of normal feeding and starvation. Analyses were facilitated by the use of a biological materials oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Each of the PCBs was rapidly removed from blood with initial storage primarily in the liver and muscle. Long-term storage was in the skin and adipose tissue with most of the stored material being unchanged compounds. Storage of metabolites in skin and adipose tissue accounted for a negligible portion of the total PCB dose. Where two adjacent carbon atoms were present, the excretion of the PCBs was, in most cases, inversely proportional to their degree of chlorination. One hexachlorobiphenyl (2,3,6,2',3',6'-) was metabolized and excreted quite rapidly. The pattern of distribution and excretion of PCBs studied in the mouse is similar to that observed in the rat except that the rates tend to be more rapid in the smaller animal.

2) Kepone is readily absorbed from the gut, stored primarily in the liver, and is slowly excreted in bile. Kepone excretion in bile is proportional to the Kepone concentration of the liver. The half-life of Kepone in the rat is approximately 18 days. The long half-life is due to the inability of the rat to metabolize Kepone at an appreciable rate and to intestinal reabsorption of much of the unmetabolized Kepone which is excreted in bile. The excretion of Kepone in bile was linear with dose between 0.1 and 10 mg/kg.

3) The gut absorption, distribution, and excretion of the major component of Firemaster BP-6®, 2,4,5,2',4',5'-hexabromobiphenyl, has been studied in the male rat. This polybrominated biphenyl was readily absorbed from the gut, initially distributed throughout the body, eventually stored primarily in the adipose tissue, was not subject to appreciable metabolism, and was excreted almost exclusively in the feces and at a very slow rate. Approximately 90% of an oral dose was absorbed from the gut and extrapolation of the rate of excretion to infinity indicates that less than 10% of the total dose would ever be excreted.

4) A mathematical model has been developed to describe the kinetics of distribution, metabolism, and excretion of 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachloro-biphenyl in rats given an intravenous dose of 0.6 mg/kg. A modified flow-limited model simulates the penta-

and hexachloro-biphenyl data for times up to 96 hours but underpredicts the mono- and dichloro-biphenyl data beyond 48 hours. The rate of metabolism by the liver decreases as chlorination increases such that the rate constant is 200 times smaller for the hexachlorobiphenyl than for the monochlorobiphenyl. The value of the biliary clearance of metabolite is nearly the same for each isomer, whereas the value of the urinary clearance decreases with increasing chlorination, being 10 times smaller for hexachlorobiphenyl than for monochlorobiphenyl. The distribution coefficients between most tissues and blood are larger for the parent compound of each isomer than for its metabolites. Hexachlorobiphenyl has the largest distribution coefficient of all isomers in each tissue, whereas the mono-, di-, and pentachloro-biphenyl show no consistent variation. For each isomer the distribution coefficient is greater in the fat than in any other tissue. Changes in the fat volume of the growing rats were incorporated in the model in order to simulate the hexachlorobiphenyl concentration in blood and fat for 42 days.

5) The mathematical model developed for the rat has been modified for extrapolation to the mouse. The model was adjusted for tissue volumes, perfusion rates, gut transport time, etc., and used to simulate the pharmacokinetics of four PCBs and their metabolites in the mouse. Predicted PCB disposition was compared with experimental data obtained at selected time points for 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachloro-biphenyl (1-, 2-, 5-, and 6-CB, respectively). Tissue and excreta concentration-time profiles obtained from the experimental data generally followed the distribution pattern predicted by the mathematical model. The disposition of these PCBs in mice was similar to that observed in rats. Peak contents in fat were about 10, 15, 40, and 75% of the dose for 1-, 2-, 5-, and 6-CB, respectively. Total excretion was inversely correlated with the degree of chlorination. Excretion in urine was significant for 1- and 2-CB (71 and 57%) but not for 5- or 6-CB. The results suggest that mathematical models based on physiological concepts can provide an operational framework for interspecies extrapolation of pharmacokinetic profiles.

6) The pharmacokinetics of 3,3',5,5'-tetrachlorobiphenyl (4-CB) was studied to determine the importance of chlorine position to the kinetics of polychlorinated biphenyl distribution, metabolism, and excretion by the rat. Tissue samples and excreta were collected at times ranging from 2 hr to 42 days after intravenous injection of a single 0.6 mg/kg dose of ^{14}C-labeled 4-CB. The relative fractions of 4-CB and its metabolites were determined in selected samples, and 4-hr bile cannulations were performed to establish the rate of biliary excretion. A mathematical model description of PCB kinetics in the rat was defined here for 4-CB and used to generate tissue and excretion concentration-time profiles for both the parent compound and its metabolites. Model predictions were in good agreement with the experimental data, and showed how the individual pharmacokinetics of 4-CB compared to several other PCBs. The rate of 4-CB metabolism and excretion was slower than would be predicted according to its degree of chlorination. The relative rates of metabolism for 4-CB and five other differently chlorinated biphenyls could be ordered according to the number of adjacent unsubstituted carbon atom pairs they contained.

It appears that chlorine position is more important than degree because the former can alter the availability of vicinal hydrogens thought to facilitate rapid PCB metabolism by mammals.

The proposed course is as follows: 1) Disposition studies have been done after treating animals with a chronic low dose until plateau levels of PCB and metabolite tissue concentration are reached. One group of animals was sacrificed at the plateau and a second group held in order to study the decay from plateau levels. The data is currently being analyzed to determine if the distribution of multiple doses was as predicted by a pharmacokinetic model based on data obtained with a single dose.

2) The amount of covalent binding (to protein and DNA) will be measured at various time points during the chronic feeding study. The amount of covalent binding will be a measure of the formation of activated metabolites, and an effort will be made to determine if the covalent binding is a function of the rate of metabolism or the type of metabolic intermediates involved.

3) Disposition studies for one more hexachlorobiphenyl will be done in the rat. From the disposition information obtained for these PCBs, structure-disposition correlations will be attempted for the PCBs in rat and mouse.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Halogenated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man.

PUBLICATIONS

Lutz, R. J., Dedrick, R. L., Matthews, H. B., Eling, T. E., and Anderson, M. W.: A preliminary pharmacokinetic model for the disposition of PCBs in rats. Drug Metab. Disp. In press.

Anderson, M. W., Eling, T. E., Lutz, R. J., Dedrick, R. L., and Matthews, H. B.: The construction of a pharmacokinetic model for the disposition of polychlorinated biphenyls in the rat. Deer Lodge Conference on Clinical Pharmacokinetics In press.

Tuey, D. B. and Matthews, H. B.: The effect of chlorine position on 3,3',5',5-tetrachlorobiphenyl in the rat. Drug Metab. Disp. In press.

Matthews, H. B., Kato, S., Morales, N. M., and Tuey, D. B.: The distribution and excretion of 2,4,5,2',4',5'-hexabromobiphenyl, the major component of Firemaster BP-6. Toxicol. Environ. Hlth. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80023-05 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Composition and Origins of the Acellular Lining Layer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Senior Staff Fellow	LP NIEHS
Other:	D. Y. Bell	Research Associate	Duke Univ.
	R. P. DiAugustine	Research Chemist	LP NIEHS
	J. D. Spalding	Research Biologist	LP NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.55	2.35	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The distal airways of the lung are lined with an acellular layer of material which appears vital in the maintenance of normal pulmonary function. The composition and origins of the acellular lining are being investigated. Current investigations have been concerned with 1) the biosynthesis and secretion of pulmonary surfactant, 2) the acellular lining as an enzymatically active medium, and 3) the protein composition of the lining.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Acellular lining material is obtained by lavaging the lungs via the trachea. Particulate materials are isolated from lavage effluents by differential and isopycnic centrifugation. Lamellar bodies, as found in type 2 cells and other subcellular organelles, are isolated from homogenates of lungs using differential centrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis, isoelectric focusing and immunoelectrophoretic procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Phospholipid transfer proteins may be involved in the biosynthesis of pulmonary surfactant. Protein(s) present in the soluble phase of homogenates of lung will facilitate the transfer of phosphatidylcholine between liposomes and pulmonary mitochondria. The presence of phospholipid transfer proteins in lung has not been demonstrated previously. The involvement of these proteins in the biosynthesis of the lamellar bodies of the type 2 cells will be investigated.

The acellular lining of the distal airways of the lungs contains a number of hydrolytic enzymes. These enzymes are generally considered to be of lysosomal origins. As much as 14% and 8% of the total lung α-mannosidase and β-N-acetylglucosaminidase, respectively, appear to exist extracellularly within the airways of the lungs. Not all lysosomal enzymes are found in this extracellular pool; for example, neuraminidase and phospholipases are not detectable and fatty acid esterase, β-glucuronidase, and arylsulphatase are barely detectable. Consequently, it appears that lysosomal hydrolases are selectively secreted into the airways of the lungs.

The major proteins of the acellular lining have been resolved using polyacrylamide gel electrophoresis. Many of the proteins present such as albumin, IgG, transferrin, and α_1-antitrypsin appear to be of serum origins; however, not all proteins present in serum are found in the acellular lining.

The origins of protein and enzymic components of the acellular lining will be investigated in detail.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The composition of the acellular lining of the lungs is complex consisting of enzymes, proteins, and lipids. Our studies indicate that many enzymic components may be secreted along with surface active phospholipids by alveolar type 2 cells; however, the major protein components appear to be selectively obtained from the plasma.

The acellular lining layer of the terminal airways is vital for normal lung function. The influence of inhaled environmental toxicants on this layer is not known, but in many environmentally related pulmonary diseases (e.g., silicoalveolar proteinosis), the composition of the acellular lining layer appears to be altered. The involvement of the acellular layer in the progression or mediation of pulmonary disease is not known. Information relating to the

composition and origins of the acellular lining layer and its variation following inhalation of toxicants is of immediate concern to occupational and environmental health.

PUBLICATIONS

Saru, S., DiAugustine, R. P., and Lynn, W. S.: Lipids in the lung lavage of patients with alveolar proteinosis and in rabbit lung lamellar organelles. Am. Rev. Resp. Dis. 114: 177-185, 1976.

Hook, G. E. R. and DiAugustine, R. P.: Secretory cells of peripheral pulmonary epithelium as targets for toxic agents. Env. Hlth. Perspect. 16: 147-156, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80028-02 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Diagnostic Markers of Pulmonary Damage and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Senior Staff Fellow	LP NIEHS
Other:	D. Nadeau	Visiting Fellow	LP NIEHS
	S. E. Fabro	Guest Worker	LP NIEHS
	D. Y. Bell	Research Associate	Duke Univ.

COOPERATING UNITS (if any) Inhalation Toxicology Section, Environmental Biology and Chemistry Branch; Dept. of Medicine, Duke University; Depts. of Obstet. and Gynecol. and Pharmacol., George Washington University, Columbia Hospital for Women

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project is concerned with the identification and characterization of components which are lung specific or pulmonary disease related which could be used as diagnostic markers for pulmonary damage or disease. Current attention is being focused on: 1) the soluble alkaline phosphatases of the normal lung, 2) abnormal hydrolases present in pulmonary lavage effluents from patients with alveolar proteinosis, and 3) enzymic components of human amniotic fluid which originate in the fetal lung.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Enzyme investigations involve the use of gel filtration chromatography, polyacrylamide gel electrophoresis, isoelectric focusing on polyacrylamide gels, immunoelectrophoresis, and differential centrifugation. Amniotic fluid is obtained during Caesarian delivery of term mothers. Pulmonary lavage effluents from patients with alveolar proteinosis is obtained as a by-product of the therapy essential to the well-being of these patients. The lavage procedure involves instillation of isotonic saline into the left or right lung of the patient followed by drainage.

MAJOR FINDINGS AND PROPOSED COURSE:
1) Markers of Pulmonary Damage. The release of organ-specific soluble aklaline phosphatases (AP) into the blood from diseased or damaged organs has prompted considerable interest in these isoenzymes as diagnostic tools. APs from a number of organs have been partially characterized, but AP of the lung has received little attention. The rabbit lung contains two soluble AP isoenzymes which have apparent molecular weights (MW) of 185,000 and 220,000 daltons. Human lung also appears to contain two APs but with MWs of 213,000 and 125,000 daltons. In both the human and the rabbit the lower molecular weight APs appear to be localized extracellularly in the airways of the lungs. These alkaline phosphatases appear to be lung-specific, at least insofar as indicated by their isoelectric points.

At present two manuscripts describing the localization and organ specificity of pulmonary alkaline phosphatases have been submitted for publication. Although these enzymes show considerable promise as markers of pulmonary damage, this aspect of the program will be discontinued since the principal investigator will be leaving this section.

2) Disease-Related Pulmonary Components. Attention has been focused on pulmonary alveolar proteinosis, a human pulmonary disease of unknown etiology but considered to be possibly of environmental origins, characterized by the accumulation of large amounts of proteinaceous material in the acini of the lungs.

The materials present in lavage effluents from the lungs of patients with alveolar proteinosis have been examined by electron microscopy, enzymatic analysis, and protein isolation procedures. Much of the insoluble materials morphologically resemble cell debris which is consistent with the presence of many degenerating and disintegrating cells. However, large numbers of myelinlike structures are present which are not found in lavage effluents from normal human or rabbit lungs. These structures appear to be characteristic of alveolar proteinosis. The soluble phase of lavage effluents contains many serum proteins such as albumin, transferrin, IgG, IgA, hemopexin, α_1-acidglycoprotein and prealbumin although many serum proteins such as the haptoglobins are absent.

Many hydrolytic enzymes are present in the lavage effluents, the most active of which appears to be a "soluble" alkaline phosphatase (AP). This enzyme is in the process of being isolated and characterized. The AP has been purified 650-fold using gel filtration and the enzyme appears to be present only as a high molecular weight complex (MW > 20 x 10^6 daltons) as judged by its exclusion from Sepharose 4B. Treatment of the complex with n-butanol or Triton X-100 results in the release of a relatively low molecular weight AP (MW ~ 200,000) which migrates as a single enzyme when electrophoresed on polyacrylamide gels. The kinetic properties of the enzyme appear similar to that present in normal human lung.

3) Pulmonary Components in Amniotic Fluid. Fetal lung-derived components have been sought in amniotic fluid obtained during Caesarian delivery of full-term infants. Phospholipid-rich lamellar bodylike structures have been isolated from the amniotic fluid. Morphologically, these structures resemble lamellar bodies of the alveolar Type II cell and lamellar body-derived structures often seen in the extracellular lining layer of adults' lungs. In normal humans lamellar bodies are found associated only with the lung, and consequently there is little doubt that the lamellar bodylike structures found in amniotic fluid have arisen from the fetal lung. Similar structures have been found in amniotic fluid from the rabbit at day 29 of gestation. As with the human, hydrolytic enzymes are found associated with the lamellar bodies of amniotic fluid from the rabbit.

The characterization of the lamellar body structures will continue and the relationship between the associated hydrolases and the ability of the fetal lung to synthesize and secrete pulmonary surfactant will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
1) Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases excepting in the advanced stages and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed most urgently.

The rabbit lung has been shown to contain two lung-specific soluble alkaline phosphatases. The presence of either of these enzymes in serum following exposure of the animal to a toxicant would be indicative of pulmonary damage.

The study of most pulmonary diseases is confined to animal models which vary enormously in their approximation to the human condition. The study of human pulmonary diseases at the biochemical level is severely restricted by the limited and limiting availability of tissues. The availability of fresh materials as by-products of patient therapy offers a unique opportunity for the study of a few human pulmonary diseases of possible environmental origin.

A number of human pulmonary diseases are characterized by the accumulation of material in the airways of the lung. The nature of these materials

and their origins are obscure. Characterization of materials which are manifestations of a disease state may provide valuable information about the processes which give rise to that particular disease condition.

2) Lamellar body-related structures derived from fetal lung and directly involved in a vital pulmonary function have been isolated from amniotic fluid. Enzymes associated with these lamellar structures should allow a simple means of estimating fetal lung maturity prior to birth.

Respiratory distress syndrome or hyaline membrane disease, which is the major cause of morbidity and mortality among premature infants, is associated with inadequate production of pulmonary surfactant. Lamellar body-related structures as found in the pulmonary airways appear to be a form of pulmonary surfactant. At present reliable tests for the estimation of pulmonary surfactant present in amniotic fluid are cumbersome since they involve chromatographic procedures. An enzyme assay for fetal lung maturity would be of major significance to clinicians as well as provide a means by which the effects of environmental pollutants on lung maturation could be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80031-01 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Role of Altered Membrane Function in Xenobiotic Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Pritchard	Research Phyiologist	LP NIEHS
	G. Neufeld	IGPA Fellow	Emporia St. Univ, Kansas

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Many freshwater and marine animals, both vertebrate and invertebrate, are particularly sensitive to certain xenobiotics. Such organisms are used as models to identify those physiological processes which are most sensitive to environmental pollutants. Particular emphasis is placed on membrane functions which underly these processes. One such model system utilizes the blue crab, which is extremely sensitive to DDT and other organochlorine pesticides, particularly at low environmental salinity where its survival depends on the crab's ability to osmoregulate. Parameters examined include the sensitivity of 1) the Na^+, K^+ ATPase to organochlorine, 2) the ion fluxes dependent upon this enzyme, and 3) the ability of the intact animal to maintain hemolymph osmolarity and ion levels.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: 1) General: to use a combination of biochemical (ATPase), physiological (ion fluxes), and pharmacological (*in vivo* toxicological) tools to establish the mechanism(s) responsible for the sensitivity of crustacea to organochlorine compounds, and then to identify the sensitive systems affected.

2) Specific: 1) To optimize assay conditions for determination of the gill Na,K-ATPase *in vitro*;, 2) to obtain baseline physiological data on the osmoregulatory role of the enzyme; 3) to determine the sensitivity of the Na,K-ATPase to organochlorine (DDT) exposure *in vitro* and *in vivo*; 4) to measure ion and water fluxes into isolated gill tissue; and 5) to quantitate changes in ion and osmotic balance in the intact animal following DDT exposure.

METHODS EMPLOYED: Na,K-ATPase is measured in freeze-dried, reconstituted homogenates of gill tissue. Ion fluxes are determined in isolated gill filaments using isotopic techniques. Hemolymph osmolarity is measured by vapor pressure osmometry and ion content determined by flame photometry.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Optimum conditions for blue crab gill Na,K-ATPase have been determined (pH 7.4, 100 mM Na^+, 10 mM K^+, 6 mM Mg^{++}). Under these conditions the K_m for ATP is 0.133 mM. In addition, a phase transition in membrane lipids at 22° is suggested by the Arrhenius plot of the temperature sensitivity of the enzyme. 2) Na,K-ATPase activity varies from gill pair to gill pair, with pairs 6 and 7 most active in both sexes. After acclimation to full-strength seawater, the specific activity of Na,K-ATPase was nearly twice as high in these gill pairs from female crabs. 3) When exposed to DDT *in vitro*, significant inhibition of Na,K-ATPase was seen at 1 ppm. Peak inhibition was reached by 9 ppm. There was little sex difference in sensitivity to DDT. Greater inhibition was observed at 19° (i.e., below the phase transition) than at 30°.

The proposed course is as follows: 1) The biochemical studies described above will be continued with primary focus on the events associated with the adaptation of the crustacean to a hypoosmotic environment. Na,K-ATPase levels normally increase during this period. The effects of DDT on the increase in enzyme activity and the sensitivity of the enzyme to DDT after salinity changes will be major concerns.

2) Studies of ion fluxes in the isolated gill and of changes in ion and osmotic concentrations in hemolymph will be performed.

3) The Ca^{++} and the Mg^{++} ATPases will also be examined in light of their sensitivity to organochlorine compounds in other systems. These enzymes, along with Na,K-ATPase, will be examined at the time of the molt when gross changes in osmolarity and calcium fluxes, in particular, are vital to the crab.

4) The Ca^{++}-ATPase and its pesticide sensitivity will be examined in the mollusc where not only its biochemistry but its involvement in Ca^{++} fluxes can be evaluated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The use of sensitive organisms may permit identification of systems particularly prone to disruption by environmental contaminants. Such studies may then a) point to systems which might also be particularly prone to damage in man and b) serve as indicators or warning systems to the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants. The sensitivity of the Na,K-ATPase shown here thus may, in conjunction with studies proposed above, not only indicate the mechanism of toxicity of organochlorine pesticides in an economically important human food, but it also suggests that the processes linked to Na,K-ATPase activity may be among the earliest and most important affected by DDT.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80032-01 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Excretion of Xenobiotics by Selected Marine and Terrestrial Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Pritchard	Research Physiologist	LP NIEHS
	J. Maguire	NIH Postdoctoral Fellow	LP NIEHS

COOPERATING UNITS (if any)

Medical University of South Carolina

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k-

Marine and terrestrial vertebrates are used to examine the role of organic ion transport in the renal and hepatic excretion of environmental contaminants such as DDT and 2,4-dichlorophenoxyacetic acid (2,4-D). Research focuses on the contribution of such transport to 1) the rate of xenobiotic depurition, 2) its toxicity to the organism, and 3) the interference of the foreign compound with elimination of endogenous wastes or toxins. The role of transport in the elimination of xenobiotics from specific organs, e.g., brain, as well as from the whole organism are also characterized.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: 1) To examine the importance of renal and hepatic organic anion transport in the rate of elimination of xenobiotics or their metabolites; 2) to assess the role of intracellular binding proteins, such as glutathione S-transferases, in transport and toxicity of organic ions; and 3) to evaluate membrane transport-related cellular accumulation in the development of the xenobiotic toxicity in target organs.

METHODS EMPLOYED: Transport of organic ions is measured in vitro using the isolated renal tubules of winter flounder and slices of rat and rabbit kidney or liver. Intracellular compartmentalization and binding are determined from efflux studies on the isolated tissue preparations after loading in vitro or in vivo. These studies are complemented by clearance measurements in the intact animals.

The binding properties of glutathione S-transferases isolated according to published procedures are determined in vitro for comparison with the intact cell data generated above.

The isolated rabbit choroid plexus is used to examine the interaction of xenobiotics with the transport of endogenous and exogenous compounds from the brain.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Studies in winter flounder, rat, and rabbit demonstrate that organic anion transport, an active membrane process, plays a major role in the elimination of DDA, 2,4-D, and similar compounds from the organism. In addition, both DDA and 2,4-D show competition in vitro and in vivo with other organic anions known to be carried by this system. Sites of such interaction include kidney, liver, and choroid plexus (see below).

2) Autoradiography shows that this transport leads to extensive intracellular accumulation of these compounds, suggesting the potential for both competition with the normal handling of other endogenous organic acids and direct cellular toxicity by the accumulated xenobiotic.

3) Efflux studies show that for the model compound DDA most of the xenobiotic (85%) is bound to cellular components. The role of such binding in determining the transcellular transport of the compound and in reducing its toxicity during transport are under investigation. Initial attention is focused upon the cytoplasmic binding proteins, such as ligandin (glutathione S-transferases).

4) Cellular effects of accumulated DDA upon the respiration of slices and isolated mitochondria from rat kidney were measured. Concentrations of 0.1 mM and above caused uncoupling of mitochondria and the appearance of negative acceptor control ratios. Accompanying the respiratory changes were altered ATPase activities (Ca^{++}, Mg^{++}, Na^{+}, K^{+}) which varied with DDA concentration in vitro. (Results of collaborative research with Drs. A. Krall and S. K. Silverthorne at the Medical University of South Carolina).

5) The results of the renal studies predicted that DDA and 2,4-D should inhibit the transport of organic acids from the brain and cerebrospinal fluid by the choroid plexus, which shows similar transport properties to kidney. Indeed, it has now been demonstrated that both compounds, at concentrations as low as 10^{-6}M (i.e., < 1 ppm), inhibit the transport of the serotonin metabolite, 5-hydroxy-3-indoleacetic acid (HIAA) by the rabbit choroid plexus in vitro. Changes in brain levels of HIAA following in vivo exposure to DDA or 2,4-D are currently being determined.

The proposed course is as follows: 1) The choroid plexus studies will be expanded to consider the dopamine metabolite, homovanillic acid (HVA), and its elimination from brain tissue in the presence of 2,4-D and DDA. Changing levels of HIAA or HVA will be monitored in response to these agents. Evidence of altered central nervous system function will be sought.

In a parallel manner, the ability of organic bases such as paraquat to alter 5-hydroxytryptamine uptake by nerve tissue will also be investigated.

2) The role of the glutathione S-transferases in the translocation of organic acids will be examined systematically using isolated, purified enzymes from liver and kidney. The approach will be to assess the interaction of organic acids with the enzyme(s) in vitro, to compare these results with the interaction of these agents on intracellular binding and transport of organic acids in intact cells, and to use pretreatment procedures to modify cellular glutathione S-transferase levels in vivo with subsequent in vitro assessment of transport, binding, and xenobiotic toxicity.

3) Since many metabolites and conjugates of xenobiotics are, like DDA and 2,4-D, organic anions, we will characterize their excretion and transport using both isolated tubule preparations in vitro and clearance techniques in vivo.

4) The basis for the uncoupling effect of DDA on oxidative phosphorylation will be examined using isolated components of the mitochondrial system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms controlling the ability of marine organisms to eliminate the many foreign compounds reaching the marine environment is vital in predicting the hazards of subsequent consumption of the species by man. Furthermore, the use of model preparations such as the isolated flounder renal tubule permits rapid assessment of the interaction of xenobiotics with renal function, or in the case of organic anions such as 2,4-D with other similar transport sites in the body. The confirmation of 2,4-D and DDA inhibition of choroid plexus transport of a normal, but toxic, brain metabolite in the rabbit is an excellent example of the predictive value of such a model system from the marine environment.

PUBLICATIONS

Pritchard , J. B.: In vitro analysis of DDA handling by rat kidney and liver. Toxicol. Appl. Pharmacol. 38: 621-630, 1976.

Pritchard, J. B., Karnaky, K. J., Jr., Guarino, A. M., and Kinter, W. B.: Renal handling of the polar DDT metabolite, DDA (2,2-bis[p-chlorophenyl] acetic acid, by marine fish. Amer. J. Physiol. In press.

Kinter, W. B. and Pritchard, J. B.: Mechanisms of Cell Injury-Altered Permeability of Cell Membranes. In Lee, D. H. K. and Murphy, S. D. (Eds.): Handbook of Environmental Physiology. Washington, Am. Physiological Society, 1977, pp. 563-576.

Pritchard, J. B., Hosterman, S., and Kleinzeller, A.: In vivo renal tubular secretion of 2-deoxy-D-galactose by the winter flounder, Pseudopleuronectes americanus. Bull. Mt. Desert Island Biol. Lab. 16: In press.

Hosterman, S. and Pritchard, J. B.: Organic acid transport: Utility of in vitro kinetic measurements to predict effectiveness of inhibitors in vivo. Bull. Mt. Desert Island Biol. Lab. 16: In press.

Jayarom, H. N., Anandaraj, M. P. J. S., Pritchard, J. B., and Guarino, A. M.: Cytotoxicity of plasma and tissue homogenates of sharks on murine lymphoblasts in culture. Bull. Mt. Desert Island Biol. Lab. 16: In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-01 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Synthesis of Polypeptide Hormones and Prohormones by Normal and Neoplastic Lung Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. DiAugustine	Research Chemist	LP NIEHS
	L. H. Lazarus	Research Chemist	LP NIEHS
Other:	I. Linnoila	Visiting Fellow	LP NIEHS
	A. Ghosh	Visiting Associate	LP NIEHS
	M. Khan	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any) Dept. of Anatomy, University of North Carolina; Depts. of Biochemistry and Pathology, Duke University; Laboratory of Molecular Biology, Burroughs Wellcome Co.; Biology Division, Oak Ridge National Laboratory

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular Pharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XX (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

These investigations focus on the potential of normal and neoplastic cells of the lung to synthesize polypeptide hormones. A pituitarylike cell which has been identified in the lungs of various species might be a stem cell and a target progenitor to lung tumors such as oat cell carcinoma. These tumors are capable of producing bioactive polypeptide hormones. One aspect of this research is to determine what specific hormones are associated with this type of cell and also to determine what regulatory role is served by such hormones in the lung.

Since human bronchogenic squamous cell carcinomas are known to produce a large form of adrenocorticotropic hormone, an investigation has been initiated to purify this material from bovine pituitary glands so that a specific radio-immunoassay might be developed and used for early detection of some bronchogenic tumors.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Radioimmunoassay, thin-layer, and column chromatography, spectrophotofluorescence (determination of corticosterone), and polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Some human tumors apparently develop from nonendocrine cells, but, yet, are capable of synthesizing polypeptide hormones. This phenomenon is generally referred to as ectopic hormone biosynthesis and endocrinelike abnormalities resulting from such tumors are called ectopic hormonal syndromes. It has been reported that human bronchogenic squamous cell carcinomas secrete a large form of adrenocorticotropic hormone (ACTH) which has negligible bioactivity. We have attempted to identify this protein in carcinogen-induced rat and mouse squamous cell carcinomas.

2) There is evidence to suggest that "big ACTH," in addition to being a probable prohormone to ACTH, might also be a precursor to other known polypeptide hormones such as β-lipotropin, β-melanocyte stimulating hormones (β-MSH), and the opioid peptides. Such possibilities will be considered should we purify the precursor molecule.

3) Oat cell and carcinoid tumors of the lung generally secrete a bioactive or "small" ACTH. Both of these types of tumors are composed of cells containing membrane-enclosed osmiophilic granules similar to those observed in the corticotrophic cells of the anterior pituitary. Such granules have been observed in small argyrophilic cells of the normal lung epithelium. We refer to these cells as lung P cells. We are attempting to further identify such cells in the pulmonary epithelium of humans and other mammals and to ascertain the presence of polypeptide hormones by using immunohistochemical methods. Pronase is being tested as a means of removing viable P cells from the epithelium. A significant advantage will be gained by establishing a culture of the cloned P cells. Such a system would allow us to study the secretion of any P-cell specific polypeptide hormone and the effects of such secretions on other lung cells, such as goblet cells.

Some major findings: 1) All four mammalian squamous cell carcinomas maintained subcutaneously contained immunoreactive ACTH. Gel filtration column chromatography of aqueous-urea extracts of one of these tumors revealed that most of the immunoreactivity was associated with a large molecular weight form of ACTH. Thus, the genetic expression of these animal tumors appears to be similar to that of the same class of human lung tumors. Biosynthesis of ACTH will be measured in cultures of mammalian lung tumors.

2) A large form of ACTH with a molecular weight of about 35,000 daltons was observed using gel filtration column chromatography of extracts of bovine pituitary glands. We shall continue efforts to purify this hormone in amounts amenable to structural analysis.

3) Argyrophilic granules have been detected in the pulmonary epithelium of the rabbit and rat. Cells containing such granules have apparently been separated from the rabbit lung epithelium following incubation of the lung airways with pronase. We are presently investigating methods to maintain these cells in culture.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polypeptide hormones secreted by endocrine organs are known to have specific physiological effects at very low serum concentrations. Numerous clinical disorders are known to be due to abnormal levels of endocrine secretions or dysfunction of complementary receptors. Identification of polypeptide hormones and proteins produced by normal lung epithelial cells is important for understanding the factors which act to regulate selective lung functions and, possibly, the function of other organs. Identification and measurement of these pulmonary factors could also be important in the assessment of damage induced by chemicals such as environmental carcinogens. Hypersecretion of cell-specific or tumor-specific polypeptides or proteins could serve as a means for a sensitive detection of neoplasia or preneoplasia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80034-01 LP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Covalent Binding of Arachidonate Metabolite(s) from Prostaglandin Synthesis to Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Pharmacokinetics Section	LP NIEHS
	M. W. Anderson	Mathematician	BB NIEHS
Other:	A. G. E. Wilson	Visiting Associate	LP NIEHS
	A. Chaudhari	Visiting Fellow	LP NIEHS
	D. J. Crutchley	Visiting Fellow	LP NIEHS
	H. J. Hawkins	Bio. Lab. Tech.	LP NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch;
Laboratory of Environmental Mutagenesis

LAB/BRANCH
Laboratory of Pharmacology; Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The goal of this project is to determine the relationships between the covalent binding of arachidonic acid metabolites produced by prostaglandin synthetase to tissue macromolecules and pulmonary toxicity. We wish to study the mechanism responsible for this covalent binding, what controls this process, and if it is of biological significance. Emphasis is being placed on what metabolite(s) binds and what regulates the covalent binding.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG) and thromboxane (TX) synthetase activity was measured in vitro using the microsomal protein from a variety of tissues and organs as an enzyme source. ^{14}C-Arachidonic acid (AA) was incubated at 37°C for various times and under several conditions. After incubation, the PG and TX were removed by solvent extraction, separated by thin-layer chromatography, and estimated by liquid scintillation techniques. Protein was precipitated by TCA and exhaustively washed with solvents. Oxygen uptake during PG and TX synthesis was measured using a Clark electrode.

MAJOR FINDINGS AND PROPOSED COURSE: We have shown that after incubation of AA with guinea pig lung microsomal protein, some radioactivity became associated with protein and some became water-soluble. Exhaustive solvent extraction and filtration through Sephadex G-25 failed to dissociate the radioactivity from protein. Indomethacin inhibited the formation of PG and TX and also inhibited the formation of covalent binding and water-soluble radioactivity. The covalent binding was dramatically reduced by the addition of glutathione (GSH) to incubation mixtures.

Analysis of the water-soluble metabolites formed on incubation of either ^{14}C-AA plus GSH or AA plus ^{14}C-GSH indicated the formation of several GSH conjugates of AA metabolites via the cyclo-oxygenase system. Incubation of ^{3}H-$PGF_{2\alpha}$, ^{3}H-PGE_2, and ^{14}C-TXB_2 with microsomal protein did not result in covalent binding or the formation of water-soluble conjugates. Thus, an intermediate(s) in the formation of TX and PG appears to be the reactive chemical responsible for binding.

Covalent binding was also observed after incubation of microsomal formation from bovine seminal vesicles, rabbit kidney, ram seminal vesicles, and platelets. The TX synthetase N-1064 did not inhibit the covalent binding with guinea pig lung microsomal protein and in some cases increased the amount of binding. The electrophile, TXA_2, which has a half-life of 30 sec at 37°C, did not covalently bind to tissue macromolecules. Thus, we concluded that the intermediate(s) responsible for the binding was not TXA_2 but could be the endoperoxide intermediate (PGG_2-PGH_2) or an unidentified reactive intermediate.

The utilization of O_2 by ram seminal vesicles during the formation for PG correlated with the covalent binding of AA metabolites to protein. The O_2 utilization was varied by changing either time of incubation or AA concentration and in both cases O_2 utilization correlated with binding. The covalent binding and O_2 consumption peaked at the identification time. At this time little or no final end-products were found in the incubation. This supports the conclusion that PGG_2 and PGH_2 are the reactive intermediates.

Covalent binding of AA metabolites to platelet microsomal protein was not inhibited by the presence of indomethacin. Indomethacin inhibited the

formation of PG and TX but elevated the metabolism of AA via a lipoxygenase system. 5,8,11,14-Eicosatetraynoic acid inhibited the formation of binding and the lipoxygenase system. Thus, a second pathway exists for the conversion of AA to reactive intermediates which covalently bind to tissue macromolecules.

The proposed course is as follows: 1) To further study the mechanism of PG and TX formation, 2) to determine which intermediate(s) covalently links to tissue, 3) to assess the potential toxicological implications of the covalent binding, 4) to prepare PGG_2 and PGH_2 and examine their interaction with tissue macromolecules, and 5) to investigate the covalent binding via the lipoxygenase system in platelets.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PG and TX are biosynthesized as the result of various stimuli. The covalent binding of an intermediate(s) formed during synthesis may be related to toxicity including mutagenesis, carcinogenesis, and teratogenesis. The TX-PG synthetase is high in pulmonary tissue and can be stimulated by environmental agents. These events could be associated with pulmonary toxicities related to environmental insults. Covalent binding of AA metabolites represents a rare case of an endogenously formed chemical reactive with tissue macromolecules.

PUBLICATIONS

Eling, T. E., Wilson, A. G. E., Chaudhari, A., and Anderson, M. W.: Covalent binding of an intermediate(s) in prostaglandin synthesis to guinea pig lung microsomal protein. Life Sci. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80035-01 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Co-oxygenation of Xenobiotics by the Prostaglandin and Thromboxane Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Anderson	Mathematician	BB NIEHS
	T. E. Eling	Head, Pharmacokinetics Section	LP NIEHS
Other:	K. Sivarajah	Visiting Fellow	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pharmacology; Biometry Branch

SECTION

Pharmacokinetics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the long-range goal of this project to study the co-oxygenation of xenobiotics by prostaglandin synthetase, particularly the pulmonary prostaglandin synthetase. The generation of toxic intermediates, carcinogens, mutagens, etc., during the co-oxygenation will be investigated. Topics of current interest are the co-oxygenation of benzopyrene during the metabolism of arachidonic acid by guinea pig lung and ram seminal vesicle microsomes. The involvement of cytochrome P-450 in this process is also under investigation.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues,, such as guinea pig lung and ram seminal vesicles, were used to examine the co-oxygenation of xenobiotics during prostaglandin synthesis. The xenobiotic metabolites were isolated from the incubation medium by extraction and by a preliminary separation by thin-layer chromatography techniques. Prostaglandin and thromboxane products were also isolated. The effect of substrate concentration, cofactors, and time on the co-oxygenation was investigated.

MAJOR FINDINGS AND PROPOSED COURSE: The carcinogen benzopyrene is oxygenated during the conversion of arachidonic acid to prostaglandins and thromboxanes by guinea pig lung and ram seminal vesicle microsomes. This co-oxygenation is particularly interesting in lung since this carcinogen enters the lung via the airways, and the release and subsequent metabolism of arachidonic acid by lung tissue is produced by numerous stimuli. Thus, exposure to benzopyrene coupled with the stimulation of prostaglandin synthesis, for example, by irritation of lung tissue, could result in the formation of toxic benzopyrene metabolites. Preliminary identification of the metabolites suggests that quinones are the major type of products produced from benzopyrene during its co-oxygenation by prostaglandin synthetase. The metabolites appear to be qualitatively similar but quantitatively different from those generated from benzopyrene by NADPH-dependent cytochrome P-450.

The proposed course is as follows: 1) To further study the co-oxygenation of benzopyrene by guinea pig lung prostaglandin synthetase; 2) to examine the co-oxygenation of other xenobiotics by prostaglandin synthetase; 3) to determine what role, if any, cytochrome P-450 has in the co-oxygenation; and 4) to begin to assess the potential toxicological implications of the co-oxygenation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many xenobiotics are thought to exert their toxicity by means of reactive metabolites which are generated *in vivo* with very short half-lives. These reactive metabolites react with tissue macromolecules to produce toxicity such as carcinogenesis, mutagenesis, teratogenesis, etc. The prostaglandin synthetase system is found in most mammalian tissues and has particularly high levels of activity in the lung and kidney. Moreover, arachidonic acid can be released from its phospholipid storage sites by various types of stimulation, for example, irritation of lung tissue by inhaled pollutants. The subsequent metabolism of arachidonic acid by prostaglandin synthetase and the simultaneous co-oxygenation of xenobiotics could produce toxic metabolites of xenobiotics.

MICHIGAN DEPARTMENT OF PUBLIC HEALTH
BUREAU OF DISEASE CONTROL AND LABORATORY SCIENCE
Lansing, Michigan 48914
(YO1-CP-60215)

TITLE: Human Health Consequences of Polybrominated Biphenyl Contamination of Farms in Michigan

CONTRACTOR'S PROJECT DIRECTOR: John W. Crenshaw, Jr., Ph.D.

PROJECT OFFICER (NIEHS): M. W. Anderson, Ph.D., Mathematician, Biometry Branch

DATE CONTRACT INITIATED: May, 1976

CURRENT ANNUAL LEVEL: $10,000

PROJECT DESCRIPTION

OBJECTIVES: This study is in its initial stages. We plan to obtain some disposition data on the subjects exposed to the polybrominated biphenyls (PBBs), i.e., blood levels and some tissue biopsies. Monitoring toxicological symptoms will be done for a long period of time (10-20 years). We are discussing possible laboratory experiments, such as pharmacokinetic studies in animal species, which would supplement the human studies.

METHODS EMPLOYED: To define and recruit a cohort of PBB-exposed individuals in Michigan and to arrange for their long-term periodic follow-up by the Michigan Department of Public Health. Blood samples and tissue specimens will be obtained periodically.

MAJOR FINDINGS AND PROPOSED COURSE: This contract is in its initial phase. Approximately 2,000 persons have enrolled for the long-term study. Approximately 1,000 blood samples have been collected. Paired blood-adipose tissue concentrations will also be obtained for some people. Previous work by the Michigan Department of Public Health has shown that high blood levels of PBBs exist in some of the exposed individuals.

Because of the suspected persistence of PBBs in the human body, long-term health monitoring of the exposed individuals is essential. Studies on the persistence of these compounds in the human body will also be done.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A large group of individuals were exposed to these chemicals. They are similar to other persistent environmental contaminants such as the polychlorinated biphenyls. Thus, it is important to collect tissue disposition and toxicologic data for scientific reasons as well as for the health monitoring of the exposed individuals.

UNIVERSITY OF ARIZONA - Tucson, Arizona 85724

TITLE: Extrapolation of PCB Deposition

CONTRACTOR'S PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): Thomas E. Eling, Ph.D., Head, Pharmacokinetics Section, LP

DATE CONTRACT INITIATED: April 15, 1977

CURRENT ANNUAL LEVEL: $194,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to obtain sufficient information on the disposition of polychlorinated biphenyls (PCBs) in dogs and monkeys and to use this knowledge in the further development of a pharmacokinetic model which will predict for a number of species the distribution, accumulation, and excretion of PCBs and other chlorinated xenobiotics after different dose levels and routes of exposure.

METHODS EMPLOYED: The distribution, accumulation, metabolism, and excretion of ^{14}C-labeled PCBs (4,4'-dichloro-, 2,4,5,2',4',5'-hexachlora-, 2,3,6,2',3',6'-hexachloro-biphenyl) will be studied in Beagle dog and Fasciculous monkey. All major tissue and excreta (urine and feces) are sampled at various times after administration of the PCB. Biliary studies are also to be conducted to determine the necessary pharmacokinetic parameters needed for modeling. Flow rate to the major organ will be determined in the monkey by means of microsphere technique.

MAJOR FINDINGS AND PROPOSED COURSE: The contract has just been awarded and the reports are not in. Our proposed course is as follows: 1) Determine the flow rates to the organs in the monkey, 2) determine the half-lives of the PCBs in dog and monkey, 3) examine the distribution of the PCBs and metabolites in various tissues in dog and monkey, 4) investigate the biliary clearance of PCBs, and 5) elucidate structures of PCB metabolites.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of PCBs in different species will help in this prediction or extrapolation of animal data to man.

RESEARCH RESOURCES PROGRAM

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

THE ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH
Summary Statement

The Environmental Biology and Chemistry Branch focuses on three objectives: to maintain collaborative support laboratories in the disciplines of Chemistry, Inhalation Toxicology, Comparative Pathology, and Veterinary Medicine; to program and coordinate experimental animal procurement, housing, and utilization within the Institute; to plan and conduct research appropriate to the capabilities of Branch laboratories.

Administratively, the Environmental Biology and Chemistry Branch is organized into four sections: Chemistry, Comparative Biology, Inhalation Toxicology, and Animal Husbandry. Operationally, specific work units (size and composition of each being determined by task requirements) often are drawn from several sections of the Branch.

ANIMAL HUSBANDRY

A major activity during the year was the completion of design and specifications for research animal quarters and support laboratories within the NIEHS permanent site. A development plan for staffing the facility, including a review of current practices is being developed; this plan will allow us to initiate long-lead activities before we occupy completed facilities.

Allocation of animal space continues to be guided by the NIEHS Animal Experimentation Committee. Lack of adequate animal space still continues to be a limiting factor in some phases of the Institute's research program. There are plans for construction and occupancy of temporary animal holding quarters in FY-78. This facility will allow us to relocate breeding colonies from within Building 15 and subsequently allocate this space for general experimentation.

Support Activities

The Animal Husbandry Section continued to develop and refine competence in a variety of technical animal procedures. The Section routinely provides capabilities in the areas of test diet preparation and feeding, animal breeding (including time-mating and maintenance of inbred stocks), as well as technical services such as administration of test chemicals, forced breeding, and necropsy assistance.

The quality of animals received at NIEHS was systematically reviewed during the year. Plans for utilizing animals of a defined flora rather than current commercial sources is being actively considered. A major concern remains that of adequate transportation from the site of production to the laboratories

in Research Triangle Park, NC. Major strides in this problem area were realized through initiating direct transport of random bred mice, rats and guinea pigs by the producers' climate controlled truck to the Research Triangle Park. Shipment of inbred mice still remains a problem.

A review of experimental animal diets used at NIEHS led to a decision to utilize an open formula rodent diet. Such a diet insures constancy of food ingredients and sources. Rigid quality control of trace contaminant levels of metals and pesticides continued to reveal NIEHS diets to be of uniformly high quality. Instituting controlled shipment from milling site to NIEHS enhanced diet quality through elimination of major transport variable associated with temperature and sanitation.

CHEMISTRY

Environmental Chemistry is the branch of chemistry which deals with the behavior of environmental agents in the environment and their affects on the physical environment and the biosphere. It has a particular focus in this Institute on delineating the chemistry of environmental agents which show (or have the potential to) biological or otherwise deleterious effects on mammalian systems as animal models for man with particular emphasis on man made (xenobiotic) persistent molecules. The Chemistry Section continues to pay particular attention to the halogenated organics and the lipophilic xenobiotics in general.

Support Activities

Environmental chemistry interfaces environmental analysis, the actual detection and measurement of environmental agents in the environment, with environmental toxicology, the detection and measurement of various deleterious biological activities of environmental agents. Therefore, chemists are frequently consulted within NIEHS in order to advise biological scientists in appropriate areas of their research. The support activities of the Chemistry Section as normal, fluctuate depending upon the resources required to meet a particular need. Chemistry support requests are progressively increasing both in terms of the number and the degree of involvement, and the requests generally fall into one of two categories: short term and to the point requests such as compound purity determinations and organic synthesis by published procedures, and a variety of long term requests which generally require new and improved methodology development. In the latter case it is difficult to determine the amount of time and effort which will be necessary to complete the task. A trial period may be necessary to demonstrate feasibility within the limitations of the group. Therefore, for obvious reasons these latter requests are carefully evaluated, and generally we seek to do this type of work on a collaborative research support basis.

As the Institute has begun to grow in anticipation of occupying the permanent facility, it has been necessary to provide a better definition of the chemistry support role. Chemistry support will generally involve the use of established known methodologies in all cases as opposed to the development of new ones. We will consider requests where new methods are required when it appears to be particularly relevant to ongoing programs within the group and/or otherwise

generally in keeping with the expertise of the group. Special considerations will be made where sufficient research priority exists to do things which can't be done on the outside at all, or only very slowly, or where it is a clear case where we can probably do it better. A number of specific routine capabilities have been identified and are available for chemistry support purposes. These services can be requested through initiation of the routine chemistry service request form. Acceptable requests are generally handled on a first come first served basis. In addition, the Office of the Head will provide support in securing procurement and research contracts of a chemical nature. Chemistry support requests are received and reviewed by the Chemistry Section Office and then passed on to one of three work groups best suited to handle the request. These work groups also form the basis for research activities within the Section. The three work groups are: Analytical Biochemistry, Bioorganic Chemistry and Synthesis, and Specialty Instrumentation. A number of other capabilities exists within the Chemistry Section which are sufficiently time consuming to require a priority collaborative research protocol to engage.

The largest number of analytical runs (over 2,000) was made by the Specialty Instrumentation group using gas chromatography-mass spectrometry (GC/MS). About 30% of these was for routine service requests, about 44% in collaborative support of various biological studies, and about 26% for instrumental and method development research. About 40% of the total runs was in support of various projects within the Chemistry Section. Nuclear magnetic resonance spectroscopy (NMR) was used on a limited number of samples for further characterization. Demands for high pressure liquid chromatography (HPLC) analysis also increased considerably and involved over 400 sample runs. The samples run included a variety of chemical standards, actual metabolites and other trace residues which occur in a milieu providing interference. One of the more involved collaborative research requests involving the Specialty Instrumentation group resulted in the identification of the metabolites of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl in the rat.

NMR was used to analyze a variety of chemical and biological metabolites and in many both ^{1}H and ^{13}C nuclei were observed using ^{1}H decoupling and fourier transform FT techniques. Most of these samples were generated from other projects within the Section. For example, using ^{13}C and ^{1}H NMR, substitution patterns of the major brominated biphenyl components of the flame retardant mixture Firemaster FF-1 were identified as 2,4,5,2',4',5'-hexabromobiphenyl and 2,3,4,5,2',4',5'-heptabromobiphenyl. Patterns of other minor isomers have also been identified in this manner. ^{13}C NMR spectra of a number of potential oxidative metabolites of benzo[a]pyrene are being obtained to aid in identification of these compounds in biological and environmental studies. The more difficult synthetic and/or preparative (using HPLC) requests included the two major brominated biphenyl components of Firemaster FF-1 and the phananthrene derivative of diethylstilbestrol and 2,4-oxazolidinedione.

A number of other requests were made for analytical services which required specific methods. These ranged from specific analysis for various enzymes, lipids, urinary calculi, silicate contents, specific isomer content, metabolites, and purity and radiopurity analysis of unlabeled and labeled research chemicals to syntheses, biosyntheses and determination of reaction rates and

reactivity profiles. More involved requests included determinations of rates of hydrolysis and reactivity toward nucleophiles for various acylating agents under teratogenicity study, purity analyses and stability studies of dosing solutions of symmetrical dimethylhydrazine and determinations of ^{35}S Captan in embryonic tissue.

Progress in development of chemical techniques and analytical methods generally useful to the Institute and their unique application to specific classes of environmental chemicals under study are continually being reviewed and evaluated. As a result of the many diverse requests, research methodology is continually being developed in analytical biochemistry and metabolism, organic chemistry and synthesis, and special instrumental analytical chemistry. Methods for the preparation, determination and characterization of various compounds and compound classes will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

Research Activities

The nature of the research in the Chemistry Section is to some degree, predetermined by its support commitment and size and space limitations. For example, the Chemistry Section attempts to provide broad areas of science competence rather than indepth and narrow science expertise. However, to do this requires the availability of diverse instrumentation which consumes space and requires routine maintenance. There are five major areas of research involvement in the Chemistry Section which are using selected classes of chemical compounds as models and/or special chemical, biochemical and instrumental techniques to develop general methodology of value to environmental health scientists:

(1) The first is seeking to develop immunoassay methods which will offer extreme specificity and sensitivity to the detection of hazardous environmental agents and their residues themselves as well as their biological insults such as mutated proteins, etc. at biologically meaningful levels.

A variety of hydrophobic aromatic halogenated haptens have been used to produce defined antigens. Antibodies to appropriately prepare antigens can generally be raised with no great difficulty. Polybrominated haptens have been shown to yield relatively ineffective antigens. Adsorbed haptens must be removed from the antigens for successful immunogenicity of the latter. Procedures for characterization and insuring the reproducibility of the antigens have been worked out. Utilization of the antisera and development of immunoassays is beginning. Sensitivity of immunoassays may be less of a problem than specificity for these types of compounds. It has also been necessary to investigate means of rapid processing of tissues and other environmental samples in regard to the unique problems associated with hydrophobic haptens.

(2) A second major effort has involved the chemical analysis and the toxicopathologic and pharmacologic effects of polychlorinated biphenyls (PCBs). In particular, the symmetrical hexachlorobiphenyls (HCBs) as

representive model PCBs with high and constant chlorine content permitting unequivocal study of a given substitution pattern have been studied in chicks and mice. Previous studies have shown separate and distinct differences in isomer toxicity. These studies have been extended to include the one remaining untested symmetrical isomer and several unsymmetrical isomers including potential metabolite possibilities and an isomer containing the least toxic and most toxic substitution pattern in one molecule, and an isomer containing the most toxic pattern in both rings and one additional ortho chlorine affecting planarity of the molecule. Differences in isomer toxicity observed can be related to chemical structure via the affects of varying chlorine substitution on compound lipophilicity and metabolism. For example, preliminary studies have established that one of the mechanisms whereby a polychlorinated biphenyl becomes bound to biological systems is through the formation of a charge transfer complex. This finding implicates aromatic amino acids and the purine bases as possible binding sites for the PCBs. Significant differences in binding propensity for two of the symmetrical hexachlorobiphenyl isomers have been demonstrated to date.

A computer assisted method for the identification of PCB isomers in a mixture requiring only minimal resolution of the mixture has been developed. Methods commonly used for the clean-up of environmental samples and tissue extracts have been evaluated relative to the quantitative determination of PCBs and chlorinated dibenzodioxins. A simple procedure for such clean-up has been proposed and supported by careful recovery studies.

The major associated impurities of PCBs, namely the chlorinated dibenzofurans and chlorinated naphthalenes, are also being investigated. Selected isomers in the chlorinated naphthalene series are being synthesized. The Diels-Alder cycloaddition reaction has been utilized in two different ways to generate the naphthalene nucleus with substituents appropriate to further transformation to selected isomers. ^{14}C Radiolabeled 2,3,7,8-tetrachlorodibenzofuran is also being synthesized for studies on species' differences in regard to variation in adsorption, metabolism, distribution and excretion. The first attempt at the radiosynthesis failed and appeared to be related to the high specific activity and accompanying radiochemistry of the phenolic precursor compound used. The synthetic reaction scheme incorporating the label in the other precursor compound, ^{14}C labeled bromobenzene, will be repeated.

(3) A third major effort is pioneering the application of chemical ionization mass spectrometry to environmental problems. Modification of our existing high pressure chemical ionization mass spectrometer has been completed which will permit the formation and observation of either positive or negative ion beams. In the negative ion mode, a considerable enhancement in sensitivity to halogenated hydrocarbons has been realized; thereby facilitating their trace level detection in biological environmental samples. Specificity has also been realized in this mode of operation when applied to the various available chlorinated

dibenzo-p-dioxins. When oxygen is used as a reagent gas, two types of product ion formation occur: the displacement of chlorine by oxygen from the molecular anion, and the formation of a quinoxide anion. The chlorine displacement reaction predominates for all isomers containing a peri chlorine. The unique sensitivity and specificity of the technique has been successfully applied to the quantitative analysis of chlorinated dibenzo-p-dioxins in dairy cow tissue extracts. These dioxins were also found in the pentachlorophenol treated lumber from the barns used to house these animals.

In the positive ion mode of operation, biacetyl was shown to be a useful reagent gas giving an acetylated product ion with a variety of functionally different organic molecules. This reagent was shown to preferentially acetylate the less sterically crowded isomer in a number of 4-t-butylcyclohexyl esters as well as norbornediol esters.

(4) A fourth major involvement continues to be the development of special instrumental analytical methodology for application to biological problems at the mechanistic (molecular) level. Currently being emphasized is the modification/update of our Fourier Transform Nuclear Magnetic Resonance (FTNMR) spectrometer. Major modifications to the XL-100 NMR system have resulted in an increase in sensitivity and extension of the capabilities of the instrument. The addition of a new magnet power supply and probe allows the use of 10 millimeter sample tubes that, in turn, results in a greater than 2 fold improvement in sensitivity for ^{1}H and ^{13}C over the previous configuration. Installation of a single side band crystal filter improved the sensitivity by an additional factor of 2 1/2. The addition of a micro insert for ^{13}C NMR has allowed routine ^{13}C NMR spectra to be obtained on 1 milligram samples and even lower for week end runs. Acquisition of the Gyro Code observe accessory extends the instruments capability to include many of the heavy metal nuclei of present day interest. Essentially, NMR spectra may be obtained for any nucleus whose frequency of absorption is within the range of 17-40 MHz. Future updates of the instrument should include accessories to extent the above range of nuclei from 7 to 40 MHz and an 18 millimeter ^{13}C probe which would allow routine ^{13}C NMR spectra to be obtained on dilute samples and biological samples of limited solubility.

^{13}C NMR has been used to characterize several chlorinated polycyclodiene pesticides and some of their phototransformation and potential metabolic products. It was shown to be superior to proton NMR for identification of these compounds and elucidation of their peculiar electronic interactions which may underlie their biological activity. In addition, NMR is being used to investigate the regiospecificity and stereospecificity of the chemical conjugation of specific ^{13}C labeled benzo[a]pyrene oxides to glutathione, enzymatically and nonenzymatically.

(5) A fifth area of involvement seeks to determine the immunologic profile of various animals under study at the Institute as a part of the dose related spectrum of toxicologic effects. Ingestion of polybrominated biphenyls appears to exert toxicologic effects in man

and animals. Results of our studies have indicated that in utero exposure to PBB in cattle induces at this particular time following exposure a state of hyperimmunity, especially with regards to cell mediated immunity. This was evidenced by a significant increase in mitogenic stimulation.

Successful treatment for exposure to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in man and animals is evidently lacking. Attempts to passively immunize mice with antibodies to 2,3,7-trichlorodibenzo-p-dioxin one day prior and one day following treatment with TCDD have failed to show any protective effect. This was evidenced by no statistically significant effect on body weight loss and mortality.

Other Activities

The Chemistry Section continues to provide unique analytical chemistry support to the study of environmental contamination problems of imminent public health concern. For example, the Institute has embarked on an epidemiological study to evaluate the health effects on infants of specific components of infant foodstuffs, particularly chlorinated pesticide residues and polychlorinated biphenyls (PCBs) in breast milk. In this regard, the Chemistry Section has reviewed and evaluated current methodologies for measuring total organic chlorine and PCB residues in body fluids and tissues. Specific methods have been identified, improved, and recommended for use by contractors in providing analytical chemistry support to this large study.

In another study, a dairy herd was found to be contaminated with chlorodibenzo-p-dioxins. Liver samples contained between 1 and 150 parts per billion octa, 0.01 and 20 parts per billion hepta, and 0.01 to 1 part per billion hexachlorodioxin. Somewhat lower levels were found in the fat.

In a third study, exposure to polybrominated biphenyls (PBBs) occurred through contaminated feed accidently shipped to two Farm Bureau Feed Mills in Michigan in 1973. The flame retardant PBB mixture FF-1 has been subjected to chemical and toxicological study. No evidence could be found for the presence of brominated dibenzo-p-dioxins or brominated dibenzofurans. The material analyzed was found to be contaminated with a few hundred parts per million bromonaphthalenes. Using chromatographic techniques, the Firemaster mixture could be separated into polar and nonpolar fractions. No evidence could be found to support the presence of highly toxic impurities in the polar fraction. The non-polar fraction was found to have toxic properties similar to those of the crude material.

The Section Office continues to evaluate the cost versus procedure benefit relationships for various custom synthesized chemicals (labeled and unlabeled) obtained on contracts. Necessary water purification equipment to guarantee a specified water quality for laboratory animal drinking water is currently being fabricated by a contractor.

Invitations to present lectures, teaching assignments at local universities, and nominations to standing committees are accepted without rancor and are usually followed by active participation. The Chemistry Section is striving

hard to establish a rapport with the various pertinent departments of the local universities in order to further enhance its research productivity and provide professional growth.

The Chemistry Section remains cognizant of the Institute safety program. A special hazardous chemical containment facility for the Section is in the fabrication stages. The facility will permit a variety of chemical operations involving hazardous chemicals to be conducted with the minimum exposure possible to laboratory personnel. The Section Office continues to cooperate with the safety office in design of specific chemical disposal procedures as needed.

COMPARATIVE BIOLOGY

The research of the Comparative Biology Section continued to focus on general toxic effects associated with halogenated polycyclic compounds such as chlorinated dibenzodioxins, chlorinated dibenzofurans, polychlorinated biphenyls and polybrominated biphenyls.

Study of the toxicity of chlorinated dibenzofurans was extended to the primate species. The LD-50 of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in the Rhesus monkey was found to be 1,000 μg/kg. The pattern of toxicity observed in the primates was identical to that previously produced by the chlorinated dibenzodioxins. The failure to produce toxicity in mice and rats with 2,3,7,8-TCDF still remains unexplained. Further studies in these species are delayed pending receipt of radio-labelled compounds being synthesized under contract. Difficulties in synthesis have occurred. Studies of the comparative toxicity of the dibenzofurans will proceed once appropriate labelled TCDF is available. Initial plans are to determine if different patterns of distribution, metabolism or absorption occur in these species.

To further investigate the mechanism of toxicity due to chlorinated dibenzodioxins, a series of experiments were performed that define the effects of TCDD on fat absorption, metabolism, and transport of nutrients from the intestine. In these studies, rats which had received a lethal dose of TCDD were found to have a reduced rate of fat absorption from the small intestine as early as 3 days post-TCDD exposure. It was further observed that the absorbed globules remained within the intestinal epithelial cells longer and by ultra-structural analysis the lipid globules are poorly transferred out of the cell. These studies are still in progress.

Previous experiments with TCDD indicated that exposure to very low doses markedly increased the susceptibility of mice to Salmonella challenge. These studies suggested that increased mortality due to Salmonella could be associated with macrophage function. Recent experiments that focused on macrophage function found no major alteration: the rate of phagocytosis was not impaired, the number of cells capable of phagocytosis and bactericidal capability of macrophage was not altered. There was, however, a decrease in the numbers of macrophages available as determined by the number of leucocytes induced to migrate into the peritoneal cavity following exposure to thioglycolate. The numerical reduction in macrophage numbers was of

sufficient magnitude to account for the increased mortality due to Salmonella challenge.

Experiments on the effect of environmental chemicals on immunocompetence expanded through study of environmental chemicals such as lead, diethylstilbestrol, and methylbutylketone. Results of previous studies with TCDD were confirmed and extended. Animals exposed prenatally and postnatally to TCDD have depressed body weights and depressed cellular immune responses. This depression is not lifelong. Responses returned to normal by 7 months of age. The T-cell mediated humoral immune responses of TCDD exposed rats is not suppressed, indicating that all T-cell subsets are not altered. In contrast, studies with lead has shown that pre- and postnatal exposure causes suppression of immune competence of both the cell mediated and humoral immunity. The studies with methylbutylketone are in progress. This work does clearly extend to a variety of chemicals the finding that environmental contaminants may exert subtle effects on man and animals, and that among these effects may be a modulation of the immune response either through suppression or enhancement.

An extensive toxicologic evaluation of Firemaster FF-1 the polybrominated biphenyl (PBB) that resulted in widespread contamination in Michigan is in progress. These studies represent a collaborative effort that involves several laboratories within the Institute. A subsequent 6 month experiment will allow for more precise characterization of toxic effects; assess the toxicity associated with chronic exposure and provide the data base from which to determine doses to be used in carcinogen bioassay performed by the National Cancer Institute.

INHALATION TOXICOLOGY

The program of the Inhalation Toxicology Section is composed of three parts: (1) Studies are conducted of compounds to which toxicologically significant exposure would be expected to be primarily by inhalation. Research is focused on expressions of toxicity at the levels of tissues, organs, and organ systems. Emphasis is placed on interactions among the cardiovascular system, the lungs, kidneys, and liver. (2) A principle effort of the Section is directed toward the advancement of inhalation technology. (3) Support is provided to intramural scientists outside the Section in the form of consultation, and collaboration, where appropriate, in matters of inhalation toxicology. The Section also allocates up to 50% of its small animal inhalation exposure chamber capacity to studies originating outside the Section as a service.

All work in inhalation research and technology during the past year has been devoted to inhalation of gases. We expect this to be extended to particulates as necessary resources become available. Studies are continuing of low molecular weight halogenated alkanes and ethers such as solvents, refrigerants, and inhalation anesthetics. The program is in the process of being expanded to include simple amines also used as solvents, as well as certain amines associated with the processing of coal and oil that might be expected to pose hazards through inhalation or ingestion.

Chronic intermittent exposure to relatively low concentrations of chlorodifluoromethane have been reported to be responsible for the appearance of superventricular arrythmias in certain occupational settings. Efforts are underway to establish an animal model of this phenomenon for the purpose of investigating the mechanism of this unique action among the halogenated alkanes. Although the conditions necessary to reproduce reliably this phenomenon in an experimental animal have not yet been completely defined, evidence for liver damage by this compound has been regularly observed.

Indocyanine green (ICG) is being reevaluated as a diagnostic agent for evaluating toxic injury to the liver. The observation that plasma ICG decay rates are relatively insensitive indicators of hepatic dysfunction in the rabbit, is consistent with the observations of others. We do have evidence, however, of an enterohepatic circulation of ICG in the rabbit which is in disagreement with the published findings of other workers. We are tentatively planning to evaluate fluorescein after the model of ICG.

Exposure of rats to 2% vinyl bromide by inhalation results in the accumulation of a pharmacologically significant body burden of inorganic bromide. The CNS depressant effect of the inorganic bromide liberated during vinyl bromide exposure is mimicked by the administration of potassium bromide in the drinking water. This observation supports the conclusion that the effect is attributable to the bromide rather than other products of biotransformation. Most of the weight loss observed during the vinyl bromide exposures was attributed to reduced feed intake rather than specific toxic effects of the compound.

Measurements of pulmonary flow resistance, compliance, and carbon monoxide capacity have been performed on several species of small laboratory animals. The magnitude of the measured variables correlated well with body weight supporting the utility of these measurements in evaluating pulmonary function in inhalation toxicology research.

The hydroxyapatite of bone is an avid sink for inorganic fluoride. The procedure has been published by others in which the femurs of mice exposed to fluoroorganic compounds are assayed for fluoride content as an index of defluorination. This method is being adapted for use in our laboratory.

A laboratory has just been completed for the study of the effects of toxic gases on the electromechanical performance of muscle, particularly myocardium. Studies are conducted on anesthetized open chested small laboratory animals, and on isolated perfused whole heart as well as tissues such as papillary muscles.

Another laboratory is being established for the purpose of studying lipoprotein surfactant, lung cells, macrophages, and a variety of enzymes associated with pulmonary lavage. This facility will be devoted to work in conjunction with the pulmonary function testing group.

A highly automated monitoring and control system for a multichamber small animal inhalation exposure facility is being built. Data representing the exposure conditions in the respective chambers are automatically acquired.

The data are used for two purposes: (1) logging and display for use by the principal investigators; and (2) control of the chambers through a feedback system. Calibration routines are employed which the operator interacts with the controller. Chamber operation is then placed under program control which includes automatic start-up, pre-programmed exposure profile generation, and shut-down of the chambers at the prescribed time. Error detection systems alert the operator to problems outside the controller's capability.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30001-06 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Health Surveillance of Laboratory Animals at NIEHS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	J. E. Thigpen	Microbiologist	EBCB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.2	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Incoming and resident animals at NIEHS are monitored for morphological abnormalities, spontaneous diseases and parasitism. The health status of research animals is vital to reliable interpretation of experimental data.

The incidence of respiratory disease is very low among laboratory rodents. Enteric pathogens have not been detected. Pinworm infections are moderately frequent but sporadic. No pathologic lesions can be attributed to these nematodes.

The incidence of pasteurellosis in NZW rabbits has been greatly reduced since purchasing them from a new source. Infectious disease in guinea pigs or hamsters has not been encountered.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: All species and strains of research animals at NIEHS are routinely observed for clinical signs of disease.

New species or strains of animals from new sources undergo complete monitoring, including examination for respiratory and enteric pathogens, ecto- and endoparasites and incidental pathology. Reference histological slides are kept on each strain or species. Pooled sera are periodically screened for viral serology.

Rodents in breeding colonies are surveyed monthly, other resident animals are monitored approximately every 8 weeks for respiratory pathogens and parasites. Tissues from major organs are collected for histopathologic examination. Fecal samples are collected weekly from individual shipping crates and examined by the flotation method for parasite eggs. Positive results confirm infestation at the supplier.

Dead, diseased or abnormal animals are usually submitted to necropsy, after clinical examination.

MAJOR FINDINGS AND PROPOSED COURSE: Incoming and resident rats monitored at monthly intervals have been free of salmonellosis and chronic respiratory disease (CRD). Occasionally, newly received weanling rats revealed focal corneal opacity and periorbital edema. This condition has proved to be resolving stages of sialodacryoadenitis, a transient viral infection. Lesions usually disappear completely within a week.

Swiss and inbred mice have been relatively free of respiratory infections. Variable titers to Sendai virus are encountered but lesions attributable to this respiratory virus are uncommon. Pinworm infections are sporadically encountered but clinical cases of diarrhea or prolapsed rectum have not been seen. The presence of this parasite is esthetically displeasing but eliminating the infection is extremely difficult to accomplish. Hamsters have been relatively disease free except for sporadic pinworm infection. Guinea pigs purchased from a reliable SPF colony have been free of infectious disease. The incidence of "snuffles" (pasteurellosis) in NZW rabbits has declined considerably since changing source of supply. During the period of 15 months (January, 1976 to March, 1977) 2,275 animals were necropsied for health surveillance. The following cases are worth mentioning, and are being prepared as case reports for publication in professional journals.

1. Chylothorax in a Rat. Chylothorax was observed in a 5-month-old rat, the mother of which was treated with a low dose (5 μg/kg body weight) of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the postnatal days 0, 7, and 14. The fluid from the chest cavity contained 25,100 leukocytes and 327 erythrocytes per mm^3. Spontaneous chylothorax in small laboratory animals, especially rodents, is very rare.

2. Heterotopic Myocardial Fibers in the Brain of a Rat. A small nodule, 0.6 mm in diameter, composed of myocardial fibers was observed in the left lateral ventricle of a rat. It was an incidental finding and may not have any clinical significance.

3. Aspergilloma in a Rat. Small "pinhead" greyish-white nodules were observed in the lung of a young female rat. Microscopically, the pulmonary nodules were composed of granulomatous tissue which contained septate branching mycelium. The fungus was identified as Aspergillus sp. by fluorescent antibody technique. Mycotic pneumonia in the rat is considered to be of rare occurrence.

4. Duplication of Lower Incisors in a Guinea Pig. An extra set of lower incisors was observed in a young male guinea pig. They were located in the right mandible producing a duplication of this portion of the jaw in association with a normal incisor. Duplication of incisors and associated portions of the mandible in guinea pigs have been reported only rarely.

Surveillance of laboratory animals will continue on a routine basis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health status of all research animals must be continually monitored; otherwise, intercurrent infections could cause misinterpretation and possible negation of valuable experimental data. In addition, microbiological and histopathological surveillance has uncovered interesting and valuable data heretofore unpublished in laboratory animal medicine.

PUBLICATIONS

Gupta, B.N.: Lymphosarcoma in a Rabbit. Am. J. Vet. Res. 37: 841-843, 1976.

Gupta, B.N.: Teratoma in a Chicken (Gallus domesticus). Avian Dis. 20: 761-768, 1976.

Thigpen, J.E., Thierry, V.L. and Gupta, B.N.: Primary Isolation of Mycobacterium avium Complex -Serotype 6 on Blood Agar. J. Clin. Microbiol. 4: 266-269, 1976.

Cunliffe-Beamer, T.L. and Feldman, D.B.: Vaginal Septa in Mice: Incidence, Inheritance, and Effect on Reproductive Performance. Lab. Anim. Sci. 26: 895-898, 1976.

Gupta, B.N. and Faith, R.E.: Chylothorax in a Rat. J.A.V.M.A. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30003-05 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of Analytical Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.W. Albro	Research Chemist	EBCB NIEHS
OTHER:	J.R. Hass	Staff Fellow	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
	K. Kohli	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)
J.K. Haseman, Ph.D., BB, NIEHS
T.A. Clemmer, Ph.D., BB, NIEHS

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.35	0.30	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This general project area has as its objective, to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which there is no analytical methodology, insufficiently specific or sensitive methodology, or methodology we are not equipped to utilize, (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: A computer-assisted method for the identification of any polychlorinated biphenyl in a mixture, requiring only minimal resolution of the mixture, has been developed. Methods commonly used for the clean-up of environment samples and tissue extracts have been evaluated relative to the quantitative determination of PCB's and chlorinated dibenzodioxins. A simple procedure for such clean-up has been proposed and supported by careful recovery studies. Methodologies will continue to be developed as needed, especially relative to halogenated compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Section as well as be of utility for other studies within the Institute.

PUBLICATIONS

Chae, K., Corbett, B.J. and Albro, P.W.: Letter to the Editor (On the Errors in Determination of PCB's by EC Detection). Environ. Health Persp. (In press).

Albro, P.W., Haseman, J.K., Clemmer, T.A. and Corbett, B.J.: Identification of the Individual Polychlorinated Biphenyls in a Mixture by Gas-Liquid Chromatography. J. Chromatogr. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30005-08 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Toxicity of Dibenzodioxins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	M. W. Harris	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

K. Chae, Ph.D., Chemistry Section, EBCB

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.6	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k-

The purpose of this study is to evaluate various dibenzodioxins with regard to the following parameters (1) dose response; (2) lethal dose; (3) clinical signs; (4) clinical pathology; and (5) anatomic pathology in guinea pigs and mice.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mice, and guinea pigs were given single or multiple doses of various dibenzodioxins (see below) orally in order to study the toxicologic, pathologic and clinical pathologic effects of the intoxication. Standard techniques were used in order to evaluate these effects.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 1-NH_2-2,3,7,8-TCDD, 1-NO_2-2,3,7,8 TCDD, 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HCDD), 1,2,3,6,7,8-HCDD, 1,2,3,7,8,9-HCDD, 2,8-dichlorodibenzo-p-dioxin, 2,3,7-trichlorodibenzo-p-dioxin (Tri CDD), 1-NH_2-3,7,8-Tri CDD, 1-NO_2-3,7,8-Tri CDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PCDD), 1,2,4,7,8-PCDD, and 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin.

MAJOR FINDINGS AND PROPOSED COURSE: The toxicopathology of the above compounds was studied in C57Bl/6gh (J67) mice and Hartley guinea pigs. The single oral LD_{50} was determined for these compounds in both species of animals. 2,3,7,8-TCDD was found more toxic than any of the other isomers. However, 1,2,3,7,8-PCDD was almost as toxic. The lesions produced by all compounds were similar in a given species. Thymic and splenic atrophy, toxic hepatitis, vascular lesions (edema and hemorrhage) and serum protein dyscrasias were the prominent features in mice. In contrast, thymic, testicular and bone marrow atrophy along with adrenal cortical hemorrhage and proliferation of the renal pelvic epithelium were more characteristically found in guinea pigs. This project is to be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known. The pattern of toxicity associated with most of the isomers has not been determined. The interactions of isomers as they relate to toxicity is unknown. These facts must be determined if assessment of the environmental health implication of products which contain dibenzodioxin isomers as contaminants are to be made.

PUBLICATIONS

McConnell, E.E. and Moore, J.A.: The Comparative Toxicity of Chlorinated Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs. Toxicol. Appl. Pharmacol. (Abstract) 37: 146, 1976.

McConnell, E.E., Moore, J.A. and Harris, M.W.: The Comparative Toxicity of Chlorinated Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs. Toxicol. Appl. Pharmacol. (In press).

McConnell, E.E., and Moore, J.A.: The Toxicopathology of TCDD. Proceed. Workshop on TCDD, Milan, Italy, October 23-24, 1976. (In press).

McConnell, E.E. and Talley, F.A.: Intracytoplasmic Hyaline Globules in the Adrenal Medulla of Laboratory Animals. Vet. Path. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 ES 30007-05 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Environmental Chemicals on Immunocompetence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Senior Staff Fellow	EBCB NIEHS
	M. I. Luster	Staff Fellow	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	C. A. Kimmell	Senior Staff Fellow	LET NIEHS
	J. A. McLachlan	Developmental Toxicologist	LET NIEHS
	M. A. Peters	Visiting Scientist	LET NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology and Chemistry Sections

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.1	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

These studies were undertaken to investigate the effects of selected environmental chemicals on the immune competence of laboratory animals. The topics of present interest include effects of transplacental exposure and effects of neonatal exposure. Chemicals investigated to date are 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), other dioxins (OCDD, HpCDD, and HxCDD, methyl mercury, lead, diethylstilbesterol (DES), and methyl butyl ketone (MBK). Results of studies completed to date show: TCDD to cause similar immunosuppression in Fischer X Wistar F_1 animals as previously seen in Fischer rats (suppression of cell mediated immune function); other dioxins to have no effect on parameters of immune function in cattle exposed in utero and during nursing through mothers exposed to dioxin contaminated pentachlorophenol treated wood; lead to suppress both cell mediated and humoral immune functions; and DES to cause humoral immune enhancement in male offspring of treated female mice.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated (T-cell) immunity is being assessed *in vitro* by the response of thymic, splenic or peripheral blood lymphocyte cultures to the mitogens Conconavalin A (Con A) or phytohemagglutinin (PHA) and *in vivo* by delayed hypersensitivity responses to tuberculin. Humoral immunity (B-cell mediated) is being assessed by the response of splenic or peripheral blood lymphocyte cultures to the mitogens pokeweed mitogen (PWM) and bacterial lipopolysaccharides, by passive hemagglutination, reverse radial diffusion and/or enumeration of plaque forming cells to measure antibody responses to both T-independent and T-dependent antigens. In addition to immunologic parameters, animals are observed for signs of general toxicity.

MAJOR FINDINGS AND PROPOSED COURSE: Results of studies with TCDD completed to date have confirmed and extended the earlier studies of Vos and Moore, Moore and Faith, and Faith and Moore. Fischer-Wistar cross bred rats respond similarly to Fischer rats in that animals exposed prenatally and postnatally have depressed body weights and depressed cellular immune responses. This depression is not life long. Responses have returned to normal levels by 7 months of age. Cell mediated responses are suppressed both as response to mitogens *in vitro* and delayed hypersensitivity responses. Humoral immune responses of TCDD exposed rats to bovine gamma globulin (BGG) are not suppressed. This indicates that TCDD does not interfere with the normal functioning of all T-cell subsets as BGG is a T-dependent antigen and requires functioning T-helper cells for a normal response.

Mice exposed to methylmercury *in utero* at days 9, 10, and 11 of gestation are now 9 months of age and are presently being evaluated for immune function.

Results of studies with lead have shown that pre- and postnatal exposure to lead causes suppression of immune competence. *In vitro* mitogen responsiveness of splenic and thymic cells to the mitogens Con A and PHA were suppressed as were *in vivo* delayed hypersensitivity responses. In addition to suppression of cell mediated immunity, lead exposure resulted in suppression of humoral immunity as shown by reduction in plaque forming cells in response to antigenic stimulation.

Studies with DES are in the preliminary stages but one very interesting finding has occurred to date. Male offspring from females treated with DES while pregnant have heightened response to T-independent antigens as exhibited by a threefold increase in plaque forming cells following injection with *E. coli* lipopolysaccharide when compared to controls. The response of these animals to sheep red blood cells (a T-dependent antigen) was normal.

The immune competence of cattle exposed to dioxins *in utero* and through nursing from mothers exposed to contaminated pentachlorophenol treated wood or exposed directly to pentachlorophenol treated wood was investigated by *in vitro* mitogen responsiveness of peripheral blood lymphocytes to the

mitogens Con A, PHA and PWM. The responsiveness of these animals was the same as that of age matched controls.

Studies with MBK are incomplete at this time. Serum samples from rats exposed to MBK and immunized with BGG (a T-dependent antigen) or dinitrophenol-lysine-ficol (a T-independent antigen) are presently being assayed for antibodies.

These studies will be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Environmental contaminants may exert subtle effects on man and animals. Among these effects may be a modulation of immune response, either suppression or enhancement. It is important to establish which environmental chemicals may induce modulation of immune responses, and their mode of action, in order to evaluate the hazards of immune modulation for man and animals.

PUBLICATIONS

Moore, J.A. and Faith, R.E.: Immunologic Response and Factors Affecting its Assessment. Environ. Health Persp. (In press).

Faith, R.E. and Moore, J.A.: Impairment of Thymus Dependent Immune Functions by Exposure of the Developing Immune System to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). J. Toxicol. Environ. Health. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30008-05 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Effects of Environmental Chemicals on Host Resistance to Infectious Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Thigpen	Microbiologist	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)

Joe Haseman, Ph.D., EBB
Histology Section, EBCB
Animal Husbandry Section, EBCB

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mouse peritoneal exudate cell concentration, cell viability, differential cell counts and on macrophage phagocytic functions. Mice were exposed to TCDD (5 µg/kg body weight) via gavage once weekly for 4 weeks and then induced with thioglycollate before collection of cells. TCDD exposed mice contained significantly less peritoneal cells than controls and differential counts revealed a reduction in the percentage of lymphocytes.

In vitro phagocytic studies showed that macrophages from TCDD exposed mice phagocytized S. bern at a reduced rate at one hour, however, at 2 and 3 hrs, they phagocytized as well as controls. No differences were found in the bactericidal ability of these macrophages. These findings indicate that subclinical levels of TCDD have a subtle, yet definite effect on the phagocytic defense system of the mouse and that the reduction in phagocytic cell numbers is the primary cause of the increased susceptibility to infection with S. bern.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Four-week old male C57BL/6JFH specific-pathogen-free mice were divided into 2 groups. Each mouse was weighed and dosed via gavage once each week for 4 weeks with either TCDD (5 µg/Kg body wt; total of 20 µg/Kg) in acetone-corn oil or only acetone-corn oil. Three days before the last dose, a leukocytic exudate was induced in the peritoneal cavity with 2.0 of thioglycollate. Five days later the mice were sacrificed and the peritoneal exudate was collected by washing the peritoneal cavity with 5 ml of HANKS balanced salt solution (HBSS) containing 10 units of heparin/ml. An aliquot from each peritoneal washing was used for determining total and differential cell counts on each mouse.

Preparation of Macrophage Monolayers: Peritoneal washings from 5 mice in each group were pooled, centrifuged and the cell pellet from each pool resuspended in 10 ml of HBSS containing 10% calf serum. Total cell concentrations and cell viability were determined on each pool. Cell concentration was adjusted to the desired numbers ($1\text{-}3 \times 10^6$) and 1 ml of this solution was added to each Leighton tube. After incubation for 1 hr at 37°C, the unattached cells were removed by washing the macrophage monolayers twice with HBSS. The resulting monolayers of almost pure macrophages were used in the phagocytic studies.

Rate of Phagocytic Uptake: The rate of phagocytosis was determined by measuring the rate at which S. bern disappeared from the extracellular medium at 0, 30, 60, 90, 120 and 180 minutes. Coverslips containing macrophage monolayers were removed at each time interval and stained to monitor the phagocytic uptake microscopically. The % of cells phagocytizing and the number of bacteria in each cell was determined.

Bactericidal Study: Macrophage monolayers were inoculated and counts were made of the total viable bacteria (both those in the supernatant and in the macrophages) at 0, 30, 60, 90, 120 and 180 minutes. Triton X-100 was used to lyse the macrophages and release the intracellular bacteria.

MAJOR FINDINGS AND PROPOSED COURSE: TCDD exposed mice contained significantly less ($P<0.01$) peritoneal exudate cells as compared to controls. In addition, % cell viability was reduced, but the differences were not statistically significant.

A significant ($P<0.05$) decrease was observed in the percentage of lymphocytes collected from the PE of TCDD exposed mice. Nonsignificant differences were observed in the percentage of other PE cells.

TCDD exposed macrophages phagocytized significantly ($P<0.05$) less S. bern bacteria at 1 hr, however, by 2 to 3 hours TCDD exposed macrophages phagocytized as well as controls.

No differences were observed in the bactericidal ability of the macrophages in either group.

These findings indicate that the numeric reduction in macrophage numbers caused by TCDD exposure in the mouse is the primary cause for the increased susceptibility to infection with S. bern.

These studies will be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of environmental chemicals when administered in sublethal doses are immunosuppressive and increased mortality rates have been observed in laboratory animals exposed to infectious agents. Humans may be exposed to these same compounds in minute quantities and subsequently may be rendered more susceptible to certain types of infectious agents. Therefore, it is important to establish why mice exposed to TCDD are more susceptible to bacterial infections.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30012-04 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparative Chemistry and Biological Properties of Selected Hexachlorobiphenyl Isomers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	J. A. Goldstein	Pharmacologist	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
	L. A. Levy	Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

Comparative Biology Section, EBCB, NIEHS, and Pesticides and Toxic Substances Lab, NERC, EPA, Research Triangle Park, North Carolina

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Previous toxicological studies using selected symmetrical hexachlorobiphenyl isomers [representing environmentally persistent polychlorinated biphenyls (PCBs) with high and constant chlorine content] in chicks and mice have been (or will be) extended to include the one remaining untested symmetrical isomer and several unsymmetrical isomers including potential metabolite possibilities, an isomer containing the least toxic and most toxic substitution pattern in one molecule, and an isomer containing the most toxic pattern and one additional ortho chlorine affecting planarity of the molecule. Differences in isomer toxicity observed can be related to chemical structure via effects of varying chlorine substitution on compound lipophilicity and metabolism.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The chemistry of the hexachlorobiphenyl (HCB) isomers was defined using a variety of synthetic, chromatographic, and spectroscopic techniques. Such biological responses as mortality, body weight change, food consumption, body organ weights, gross and microscopic pathology, incidence of edema and porphyria, liver enzyme induction, etc. were measured simultaneously. Chromatographic and spectroscopic techniques were also used to isolate and identify metabolites. Several of the desired HCB isomers were obtained through total synthesis in our laboratories.

MAJOR FINDINGS AND PROPOSED COURSE: Studies of four symmetrical HCB's (3,4,5,3',4',5'; 2,4,6,2',4',6'; 2,4,5,2',4',5'; 2,3,6,2',3',6') in male mice have been completed and the major toxicopathologic effects observed were similar to those found in previous studies with chickens. Differences in isomer toxicity can be related to chemical structure via effects of varying chlorine substitution on compound lipophilicity and metabolism. Preliminary studies in chicks with the only remaining untested symmetrical HCB (2,3,5,2',3',5') indicates that it may be the least toxic isomer of all showing no significant effect on any of the parameters studied. A slightly higher body weight gain with lower food intake remains to be explained. Preliminary studies in chicks with 2-hydroxy-3,4,5,3',4',5'-HCB, a potential metabolite of the most toxic isomer (3,4,5,3',4',5') studied along with another unsymmetrical hydroxylated HCB (5-hydroxy-2,3,4,3',4',5') do not support the possibility that high toxicity is mediated through hydroxylated metabolite intermediates. Metabolism and pharmacokinetic studies using the more interesting ^{14}C-labeled HCBs are in progress and will be described elsewhere. Such studies are providing useful information for explaining differences in biological properties of these isomers. We propose to investigate the toxicity of the 2,3,5,2',3',5'-isomer and two unsymmetrical isomers, viz. 2,3,5,3',4',5' (containing the least and most toxic patterns in one molecule) and 2,3,4,5,3',4',5' (containing the most toxic pattern and one additional ortho chlorine affecting planarity of the molecule) in the mouse before completing this project, and an isostere of the most toxic isomer, viz. 2,2-difluro-3,4,5,3',4',5'.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use and occurrence of the polychlorinated biphenyls has necessitated investigations of their possible human health effects. In order to facilitate such studies, systematic examination of purified isomers is necessary to obtain clarity and definition of the biological effects. The HCBs with high chlorine content represent an environmentally persistent class of PCBs. Symmetrical HCBs with identical chlorine substitution in each half of the biphenyl molecule permit unequivocal study of the biological effects of a given substitution pattern.

PUBLICATIONS

Biocca, M., Gupta, B.N., Chae, K., Moore, J.A., McKinney, J.D.: Toxicity of Selected Symmetrical Hexachlorobiphenyls in Male Mice. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30015-03 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies in Chemical Ionization Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. R. Hass Staff Fellow EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

Department of Chemistry, University of North Carolina, Chapel Hill, NC 27514

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.1	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Previous findings concerning the gas phase ion chemistry of biacetyl under ion cyclotron resonance (icr) were extended to chemical ionization (CI) conditions. Steric effects for gas phase acetylation reactions were noted.

Modifications of CI mass spectrometer to sample negative ions were completed. The negative CI mass spectra of the available chlorodibenzo-p-dioxins were obtained using oxygen, methane/oxygen and nitrogen/oxygen as the reagent gas. Selected environmental samples were analyzed by negative CI for dioxin content.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromatography/mass spectrometry techniques, standard chemical ionization mass spectrometry techniques, negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Biacetyl was shown to be a useful reagent gas for positive ion chemical ionization mass spectrometry giving an acetylated product ion with a variety of functionally different organic molecules. This reagent was shown to preferentially acetylate the less sterically crowded isomer number of 4-t-butylcyclohexyl esters as well as norbornedicol esters. This project is complete.

The methane/oxygen and nitrogen/oxygen negative CI mass spectra of the available chlorodibenzo-p-dioxins exhibit primarily loss of H, Cl, Cl_2 and various combinations from the moleculer anion with the methane/oxygen giving somewhat higher sensitivity than nitrogen oxygen. Oxygen as a reagent gas lead to two types of product ion: the displacement of chlorine by oxygen from the molecular anion and the formation of a quinoxide anion. The chlorine displacement reaction predominates for all isomers containing a peri chlorine. We propose to extend this work to study the negative ion CI mass spectra of chlorinated environmental hazards.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The application of mass spectrometry to problems in biomedical analysis in recent years has been particularly fruitful. Chemical ionization techniques have provided information complementary to data acquired using electron impact ionization methods. This project should allow a means to gather further information concerning the nature of an unknown sample.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30018-04 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ester Hydrolases at the Environmental Interface

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. W. Albro Research Chemist EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this project is to evaluate the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. The major lipase catalyzing hydrolysis of phthalate esters, herbicide esters (e.g. 2,4-D esters) etc. has been isolated from rat pancreas and characterized chemically and mechanistically. Efforts are under way to procure sufficient purified enzyme for use in studies of protein-ligand interactions.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic rubrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbamates.

MAJOR FINDINGS AND PROPOSED COURSE: The nonspecific lipase of human plasma is able to hydrolyze the phthalate plasticizer DEHP as it leaches into stored blood from blood bags, even at 4°C. Nonspecific lipase activity does not appear to a detectable extent in human lung lavage fluid. We propose to further investigate xenobiotic-hydrolyzing lipases from lung and skin, and to institute detailed studies of enzyme-ligand binding using purified enzyme from pancreas.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never previously been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

PUBLICATIONS

Albro, P.W., Corbett, B.J. and Latimer, A.D.: Purification and Partial Characterization of Nonspecific Lipase from Rat Pancreas. Biochim. Biophys. Acta 424: 351-365, 1976.

Albro, P.W., Corbett, B.J. and Hass, J.R.: The mechanism for Nonspecific Lipase from Rat Pancreas. Biochim. Biophys. Acta 431: 493-506, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30020-06 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transport and Metabolism of Phthalate Esters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	J. R. Hass	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

C. C. Peck, M.D., LTC, MC, LAIR
D. Odom, Ph.D., CPT, MC, LAIR

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER (by NIEHS)

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Previous studies of the adsorption, transport, distribution, metabolism and excretion of phthalate esters in the rat have been extended to include uptake of phthalates into human blood stored in blood bags, and metabolism and pharmacokinetics of di-(2-ethylhexyl)phthalate (DEHP) in the African Green monkey.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard enzymology techniques, radiotracer (^{14}C)$_{13}$accountability, GC-Mass Spectrometry with multiple ion monitoring; use of ^{13}C-label to eliminate environmental contamination interference.

MAJOR FINDINGS AND PROPOSED COURSE: Metabolites of DEHP in monkey urine are quite different from those in rat urine, and much closer to those seen in human urine. Human blood stored in PVC blood bags contains not only DEHP, but also mono-2-ethylhexyl phthalate, the toxicological significance of which has not been evaluated. We propose to examine the metabolism and pharmacokinetics of DEHP and the monoester (MEHP) in samples obtained at LAIR from human volunteers.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Determination of biochemical parameters related to the toxicological significance of DEHP and MEHP in human blood is relevant for establishment of limits on storage time in PVC blood bags. DEHP is, in addition, a ubiquitous environmental pollutant, exposure to which includes the relatively unexplored routes of absorption through the skin and inhalation. Uptake by these routes could yield tissue distribution and toxicological significance different from that seen on oral or intravenous exposure.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30026-03 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Histopathologic Effects of Euthanasia on Laboratory Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The method of euthanasia may alter the normal morphology of organs and cause misinterpretation of histopathologic findings. This study was undertaken to study the histopathologic changes caused by various methods of euthanasia in rats, mice, guinea pigs and rabbits. Lesions resulting from a particular method of euthanasia were consistent from species to species. Each method studied affected the lungs to some degree, ranging from mild congestion of alveolar capillaries to marked intramural edema of pulmonary arteries and alteration of vascular permeability. Euthanasia of experimental animals by overexposure to CO_2, or intraperitoneal injection of concentrated sodium pentobarbital seemed most suitable for pulmonary studies. Decapitation (mice, rats, guinea pigs), cervical dislocation (mice), CO_2 and intracardial injection of sodium pentobarbital were more suitable for examination of abdominal viscera.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Groups of 4 animals of each species were killed according to commonly employed methods which included decapitation (mice, rats, guinea pigs), cervical dislocation (mice), CO_2, air embolism (rabbits) and various parenteral injections of sodium pentobarbital. Animals were necropsied immediately after death and tissues were processed for microscopic examination.

MAJOR FINDINGS AND PROPOSED COURSE: Most methods of euthanasia allowed good visualization of abdominal viscera, except concentrated sodium pentobarbital injected intraperitoneally. This drug caused necrosis of the serosal surface of underlying organs. Most methods of euthanasia caused variable pulmonary vascular congestion but usually the lung architecture was not distorted. Euthanasia with CO_2 or sodium pentobarbital injected intraperitoneally or intravenously affected the lungs minimally. Concentrated sodium pentobarbital injected into the right ventricle of the heart caused hemolysis of erythrocytes and edema of both pulmonary arterial walls and alveoli. This project has been completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The method of euthanasia merits careful deliberation in the design of experiments. If histopathologic interpretation must be made, it is important that euthanasia does not distort cellular architecture.

PUBLICATIONS

Feldman, D.B. and Gupta, B.N.: Histopathologic Changes in Laboratory Animals Resulting from Various Methods of Euthanasia. Lab. Anim. Sci. 26: 218-221, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30028-06 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Evaluation of a Captive-Born and Reared Colony of Opossums

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. B. Feldman Veterinary Medical Officer EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

A closed breeding colony of Virginia opossums (Didelphis marsupialis virginiania) is maintained to provide neonates for research purposes. Opossums are bred during the 4 month breeding season (January-April) by establishing natural matings within a group of 2-3 females to 1 male. Mortality is high among males who are killed by the females. The conception rate in 1976 was 22% (13 litters from 49 matings) as compared to 43% in 1975. This continual decline in conception over the past several years suggests that subtle environmental and/or husbandry factors adversely affect fertility of captive born-reared opossums. The colony was discontinued in November, 1976 and the animals were shipped to Germany to establish breeding colonies.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: During the 1976 breeding season (January-May), opossums were bred by conventional means i.e. natural matings within a group of 2-3 females:1 male.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 13 litters were born as a result of breeding 49 females (22% conception rate). The number of young totaled 96 (average litter size 7.3) of which 51 survived to weaning age. In November, 1976 the colony was discontinued. Most of the opossums were shipped to Germany for the purpose of establishing breeding colonies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This colony has been monitored from year to year as a feasible means of providing sufficient animals for research. During the past year research utilizing the opossum has been considerably reduced and the concomitant demands for neonates has lessened. Because of the value of those opossums, the colony will probably be perpetuated but only a minimal number of breeding opossums will be maintained from year to year. In the event of increased demand, the colony can be expanded.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30030-02 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Defined Diet Related Hydronephrosis and Hydroureter

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Senior Staff Fellow	EBCB NIEHS
OTHER:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Initially a high incidence of hydronephrosis and hydroureter was observed in CD rats fed a marginally zinc deficient defined diet in which sucrose was the sole sugar. These studies were undertaken to elucidate the etiology of the urinary tract pathology. Presently sources of carbohydrate and zinc levels in difined diets are being investigated in relation to urinary tract pathology.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Three week old CD rats, both male and female, were randomly assigned to various diet groups. Diets employed to date have differed in zinc content and in sugar source (either sucrose or dextrose). Body weight gains, food consumption, and water consumption were monitored three times weekly. At the end of three weeks the animals were sacrificed and complete necropsies performed.

MAJOR FINDINGS AND PROPOSED COURSE: An attempt was made to repeat earlier studies with sucrose diets. The results were equivocal. No gross lesions developed but there were indications of microscopic changes. Presently animals are being fed sucrose containing diets in an attempt to repeat earlier findings.

Further investigations planned include: repeating these studies in one or more other strains of rats and in other species such as mice and hamsters in order to develop a feel for strain and species susceptibility; investigation of a diet utilizing a sugar source of fructose and dextrose; and investigation of diets utilizing other disaccharides.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many studies at this Institute and other institutions require the use of defined diets. It is, therefore, of importance to know the effects of defined diets on experimental animals, especially if one or more components of the diet produce pathological changes. In addition, pathological changes that are diet related are of interest from a nutritional point of view.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30034-02 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemistry of Chlorinated Aromatic Compounds and Their Environmental Transformation Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: L. A. Levy Research Chemist EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

The development of rational synthetic routes to halogenated naphthalenes, chlorinated biphenyls and especially the assumed reactive intermediate arene oxides have been investigated. Models appropriate to the study of the chemical and physical properties of these classes of compounds have been prepared.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: The Diels-Alder cycloaddition reaction has been utilized in two different ways to generate the naphthalene nucleus with substituents appropriate to further transformation. Both of these approaches utilized a ortho xylylene synthon, generated from different precursors, which was allowed to undergo cycloaddition with different dieneophiles to produce the naphthalene nucleus. These derivatives have led to chlorinated and brominated naphthalenes as well as intermediates with the potential to be further elaborated to other halogenated naphthalenes and their possible metabolites.

Further developmental work on the problem of the synthesis of biphenyl oxides has provided methods suitable for the large scale production of intermediates appropriate for the final synthetic steps.

Some chlorinated phenanthrenes have been prepared. This was done to consider the question of toxicity as a function of structure and as models to further probe the nature of the interactions of ortho-ortho prime chloro substituents in biphenyls. Some questions about the fragmentation observed in the mass spectra of PCB's which were raised in earlier work may be answered from the study of these phenanthrene derivatives, which incorporate a constrained biphenyl unit within their structure.

To continue the synthesis of individual isomers of halogenated naphthalenes as required as well as their potential or actual metabolites such as arene oxides and hydroxylated derivatives is proposed. Continuing attention is to be focused on the use of the cycloaddition reaction and associated sequence mentioned above. The preparation of appropriate models to more fully explore the chemical and spectral characteristics of the molecules will be carried out as time permits.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCB's and halogenated naphthalenes are known to be complex mixtures and are recognized to be of major environmental importance due to their widespread ecological distribution and their concentration in the food chain. Furthermore, chlorinated dibenzofuran impurities found along with PCB and/or produced from PCB by natural processes are known to be more toxic. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit more definitive biological and toxicological studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30035-02 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemical Techniques and Spectroscopic Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
	P. A. Albro	Research Chemist	EBCB NIEHS
	J. R. Hass	Staff Fellow	EBCB NIEHS
OTHER:	K. C. Chae	Staff Fellow	EBCB NIEHS
	R. H. Cox	Visiting Scientist	EBCB NIEHS
	L. A. Levy	Research Chemist	EBCB NIEHS
	M. I. Luster	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

NCI Chemical Repository.
Others as needed.

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	1.6	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This general project has as its objective to employ chemical techniques, both analytical and synthetic, and spectroscopic methods, in particular gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy (NMR) and high pressure liquid chromatography (HPLC), to the preparation, determination and characterization of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general compounds covered by this study will include those for which adequate techniques and methods are already available or can be adapted.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytical methods emphasized in this study are GC/MS, NMR or fourier transform NMR and HPLC. A variety of synthetic techniques are employed. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: The GC/MS facility made the largest number (over 2000) of sample runs for analysis. About 30% of these was for routine service request, about 44% in collaborative support of various biological studies, and about 26% for instrumental and method development research. About 40% of the total runs was in support of various projects within the Chemistry Section.

The number of requests for high pressure liquid chromatography (HPLC) analysis also increased considerably and involved over 400 sample runs.

One of the more involved collaborative research requests resulted in identification of the metabolites of 4-chlorobiphenyl in the rat as 4'-hydroxy-4-chlorobiphenyl, 3'-hydroxy-4-chlorobiphenyl and 3'-methoxy (or hydroxy)-4'-hydroxy (or methoxy)-chlorobiphenyl. The metabolites of 4,4'-dichlorobiphenyl were also identified as 4-hydroxy-3',4-dichlorobiphenyl and 2-hydroxy-4,4'-dichlorobiphenyl. In support of other studies, the major metabolite of kepone in the rat was isolated using high pressure liquid chromatography. Attempts at characterization by mass spectrometry have thus far failed. The GC/MS characterization of metabolites of the symmetrical hexachlorobiphenyls in chicks was completed. A careful examination of the material originally fed to the chicks indicated that trace quantities of iodopentachlorobiphenyls were present which could account for "anomalous metabolites" previously found in their excreta.

The NMR facility handled a number of different samples including a variety of chemical and biological metabolite and in many observed both ^{1}H and ^{13}C nuclei using ^{1}H decoupling and fourier transform (FT) techniques. Most of these samples were generated from other projects within the section. For example, using ^{13}C and ^{1}H NMR the substitution patterns of the major brominated biphenyl components of the flame retardant mixture, Firemaster FF-1, were identified as 2,4,5,2',4',5'-hexabromobiphenyl and 2,3,4,5,2',4',5'-heptabromobiphenyl. Patterns of other minor isomers have also been identified in this manner. ^{13}C-NMR spectra of a number of potential oxidative metabolites of benzo[a]pyrene will be obtained to aid in identification of these compounds in biological and environmental studies.

A number of other requests were made for analytical services which required specific methods. These ranged from specific analyses for various enzymes, lipids, urinary calculae, silicate content, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to syntheses, biosyntheses and determination of reaction rates and reactivity profiles. The more involved requests included determinations of rates of hydrolysis and reactivity toward nucleophiles for various acylating agents

under teratogenicity study, purity analyses and stability studies of dosing solutions of symmetrical dimethylhydrazine and determinations of ^{35}S-Captan in embryonic tissues.

The more difficult synthetic and/or preparative requests included the two major brominated biphenyl components of Firemaster FF-1, the phenanthrene derivative of diethylstilbestrol, and 2,4-oxazolidinedione, all for biological study.

The state-of-the art in chemical techniques and analytical methods development generally useful to the Institute or the application to specific classes of environmental chemicals under study is continually being reviewed and evaluated. New and improved techniques and methodologies will be employed as needed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

PUBLICATIONS

McKinney, J.D.: Analysis of TCDD in Environmental Samples. Proceedings of the Conference on Chlorinated Phenoxy Acids and their Dioxins. Ecological Bulletins, Swedish Research Council. (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30038-02 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Toxicity of Dibenzofurans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	M. W. Harris	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

K. Chae, Ph.D., Chemistry Section, EBCB
Hazleton Research Laboratories, Vienna, VA

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study is to evaluate various dibenzofurans with regard to the following parameters (1) Dose response; (2) Lethal dose; (3) Clinical signs; (4) Clinical Pathology; and (5) Anatomic Pathology in guinea pigs, mice and monkeys.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Male guinea pigs and/or mice were given single or multiple doses of various dibenzofurans [2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,7,8-tetrabromodibenzofuran (TBDF), and 2,3,4,7,8-pentachlorodibenzofuran (PCDF)] orally. Rhesus monkeys were given single oral doses of TCDF. Standard techniques were used to evaluate the objectives.

MAJOR FINDINGS AND PROPOSED COURSE: These compounds appear to be extremely toxic with the LD_{50} in guinea pigs being 5-10 µg/kg for TCDF and 3-10 µg/kg for PCDF or about 3 to 5 times less toxic than their dioxin counterpart. The brominated form (TBDF) was of about the same toxicity (3-10 µg/kg) as TCDF. The LD_{50} of TCDF in Rhesus monkeys was 1000 µg/kg. The lesions produced by these compounds were similar in a given species of animal. However, a distinct difference was noted between species. Thymic and splenic atrophy were noted in all species. Few lesions were found in mice because it was impossible to give a high enough single oral dose (>6000 µg/kg) to cause significant toxicity. Testicular and bone marrow atrophy along with adrenal cortical hemorrhage and hyperplasia of the renal pelvic epithelium were more characteristically found in guinea pigs. Many of the above lesions were observed in monkeys. In addition, cutaneous lesions of the eyelids, face, ear canal and toe nails were also apparant. Except for the mouse, the lesions were comparable to those produced by dioxins in the same species of animal. This project is to be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated dibenzofurans may occur in association with useful chlorinated cyclic compounds. In addition, there is evidence that they were contaminants of polychlorinated biphenyls and may have contributed in the Yusho accident. The pattern of toxicity associated with most of the isomers is not known. These facts must be known if assessment of the environmental health implication of the materials which contain these compounds is to be made.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 ES 30039-02 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Monkeys (Macaca mulatta)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	D. Dalgard	Project Coordinator	Hazleton, Labs, Vienna, VA

COOPERATING UNITS (if any)

K. Chae, Chemistry Section, EBCB

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study was to establish the oral lethal dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in monkeys (Macaca mulatta). In addition to evaluate the following parameters (1) clinical signs; (2) clinical pathology; and (3) anatomic pathology.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Monkeys (at Hazelton Labs) were given a single dose of TCDD in corn oil orally. Parameters of evaluation included clinical observations, hematology and clinical chemistry (pre and post exposure), macro and histopathology.

MAJOR FINDINGS AND PROPOSED COURSE: The LD_{50} was less than 70 µg/kg, probably around 50 µg/kg. Time to death was dose dependent (28 ± 5 days at 350 µg/kg and 42 ± 5 days at 70 µg/kg). The clinical appearance of the animals was characterized by weight loss, focal alopecia, blepharitis, acne, abnormal nail growth and progressive weakness. Abnormal hematologic findings included mild anemia, neutrophilia and lymphopenia. There was a decrease in serum cholesterol but an increase in triglyceride levels. Necropsy and histopathologic examination revealed metaplastic and hypertrophic lesions in sebaceous glands, especially in the eyelids and ear canals. There was marked depletion of lymphoid elements, especially in the thymus where only a few medullary cells remained. The stomach, duodenum and colon showed early cystic and hypertrophic changes in the crypts. The liver showed milk hypertrophy and hyperplasia of the biliary system but other than this it was relatively normal. The biliary lesion extended into the gall bladder and common bile duct where it was much more severe. Hyperplasia of the renal pelvic epithelium was also noted. The liver, adrenal gland and kidney showed an increase relative to body weight while the thymus was dramatically reduced in size. This project is completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known as has been demonstrated in chickens, guinea pigs and small rodents. This primate evaluation is a natural outgrowth of these studies.

PUBLICATIONS

McConnell, E.E., Moore, J.A. and Dalgard, D.W.: Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rhesus Monkeys (Macaca mulatta) Following a Single Oral Dose. Toxicol. Appl. Pharmacol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30040-02 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Dose Response to the Carcinogens, 1,2-Dimethylhydrazine (DMH) and Methylazoxymethanol (MAM) in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	R. E. Wilson	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

J. Fawkes, Chemistry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The "HEW Committee to Coordinate Toxicology and Other Related Programs" has designed a study to establish the biologic consequence from ingestion of asbestos. NIEHS has the responsibility for effecting these protocols. One aspect of the study is to determine if asbestos can act as a co-carcinogen, specifically in the gastrointestinal tract. 1,2-Dimethylhydrazine (DMH) and methylazoxymethanol (MAM) are known intestinal carcinogens, however, the dose required to produce a relatively low incidence (15 ± 5%) of colon cancer is not known. The objective of this study is to derive this information and in addition, to critically evaluate any neoplasm produced as to location, size, and morphology.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Five week old male and female Fischer (CDF) rats were given various amounts (30, 15, 7.5, 4, 1.0 and 0.2 mg/kg) of either DMH or MAM via gavage. They were dosed every other week for 8 weeks (5 doses). Body weight gain was monitored. The study was terminated at 12 to 14 months after the first dose. The incidence of neoplasia and latent period was recorded. Tumors were recorded as to their location, number, size and morphology.

MAJOR FINDINGS AND PROPOSED COURSE: There was a small but significant dose related decrease in weight gain in the 30 mg/kg animals (both compounds). As of April 1, 1977 the histopathologic results are not complete. However, the macroscopic findings suggest that the dose of DMH required to produce a 15-25% incidence of colon neoplasia is 15 mg/kg in female and 7.5 mg/kg in male rats. The data is less clear and less complete with MAM but the dose required to produce the same incidence of colonic neoplasia should be about the same. However, there were more tumors in other locations with MAM. For this reason, DMH was considered a better model for use in the asbestos study. Project will be completed this FY.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Colon cancer is the 2nd leading form of neoplasia in men and 3rd in women. Various studies have indicated that it is probably environmentally related. Suggestions have been made that asbestos may play a role in the causation. The development of a method which would yield a relatively low incidence would serve as a useful model for the study of this important disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30041-02 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Hydrophobic Haptens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	M. I. Luster	Staff Fellow	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
	A. Chaudhary	Visiting Fellow	EBCB NIEHS
	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, EBCB

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	3.1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A variety of hydrophobic, aromatic, halogenated haptens have been used to produce defined antigens. Antibodies to many of these have been raised successfully. Procedures for characterization and insuring reproducibility of the antigens have been worked out. Utilization of the antisera in development of immunoassays is beginning. It is hoped that these assays will provide relatively inexpensive, specific and sensitive alternatives to assay methods presently available, as well as provide confirmatory techniques.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques: condensation reactions, amination, reduction, mixed ester formation, active ester formation, convergent synthesis, spectroscopic and chromatographic characterization. Conjugation techniques: diazo coupling, mixed anhydride acylation, active ester acylation. Characterization of conjugates by UV spectroscopy, gel filtration, amino acid analysis, NMR spectrometry, and chemical assay of functional groups. Antibody production methods: standard procedures, with adjuvant, in rabbits. Antibody assay methods: double immunodiffusion, fluorescent antigen, radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: Antibodies to appropriately prepared antigens can generally be raised with no great difficulty. Polybrominated haptens yield relatively ineffective antigens. Adsorbed haptens must be removed from the antigens for successful immunogenicity of the latter. Sensitivity of immunoassays may be less of a problem than specificity with these types of compounds. We propose to continue immunoassay development, striving for maximum specificity and picomole sensitivity. Also, it is necessary to investigate means of rapid processing of tissues and other environmental samples, in regard to the unique problems associated with hydrophobic haptens.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Chae, K., Cho, L.K., and McKinney, J.D.: Synthesis of 1-Amino-3,7,8-Trichlorodibenzo-p-dioxin and 1-Amino-2,3,7,8-Tetrachlorodibenzo-p-dioxin as Haptenic Compounds. J. Agric. Food Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30042-02 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Contaminants on Cardiac Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBCB NIEHS
OTHER:	M. P. Moorman	Biomedical Engineer	EBCB NIEHS
	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS

COOPERATING UNITS (if any)

Pathology Unit, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.40	0.25	0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This project is directed toward the detection of functional changes in the cardiovascular system associated with the inhalation of anesthetics, fluorocarbons, and brominated compounds as well as energy-related pollutants. The function of isolated, perfused rabbit, rat, and guinea pig hearts will be monitored. Animals may be previously exposed to contaminants followed by excision and perfusion of hearts or the hearts may be exposed acutely in vitro. Myocardial mechanical, electrical, and metabolic activity may be monitored concurrently.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In vivo and in vitro model systems are used. Rabbits are exposed to 6% $CHClF_2$ for 6 hr/day, 5 days/wk. Exposures were continued for 3 months in 3 experiments and 9-12 in a 4th experiment.

In vitro, two model systems are employed: (1) the perfused, isolated, Langendorff heart preparation; and (2) the superfused isolated papillary muscle preparation. Mechanical activity in the whole heart is monitored using an intraventricular ballon connected to a pressure transducer, and in the papillary muscle using a force transducer. Electrical activity is assessed using surface electrograms or microelectrode recordings. Metabolic activity of the whole heart is determined by monitoring perfusate pH, PCO_2, and PO_2 as it enters the aorta and as it exits the coronary sinus.

MAJOR FINDINGS AND PROPOSED COURSE: Supraventricular arrhythmias that persisted after cessation of exposure, and after the passage of enough time to reduce the total body burden of the compound to undetectable levels, were detected in one of 14 rabbits exposed for 6-8 weeks. Studies are continuing in an effort to determine if this was a spurious occurrence, represented a genuine rare response to the presence of the compound, or if the conditions of the experiment were not favorable for development of the functional abnormality in more individuals.

Incidental to these findings was the observation that in all animals, after 4 wk exposure, serum enzyme levels began to rise, suggesting a hepatotoxic response to the compound. Based on the degree to which the enzyme levels increased and histopathological observations, the conclusion was reached that the liver changes would be expected to be reversible. This hypothesis will be tested.

The performance of the in vitro experiments has been deferred pending the development of the appropriate in vivo model.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung represents a "short circuit" to the heart since inhaled materials which are transferred to the blood are then pumped directly to the heart and to the blood supply to the heart without benefit of passing through any of the detoxification organs such as the liver and the kidney. Thus, it is necessary that we measure the cardiac effects of these agents directly. Many halogenated alkanes are known to be cardiac depressants, causing arrhythmogenic and or negative inotropic effects. Since the cardiac effects could be lethal, it is necessary to establish an understanding of the mechanism whereby these agents cause their cardiotoxicity.

PUBLICATIONS

Back, K.C. and Van Stee, E.W.: Toxicology of Haloalkane Propellants and Fire Extinguishants. Ann. Rev. Pharmacol. 17: 83-95, 1977.

Van Stee, E.W., McConnell, E.E., Patel, J.M., Hamlin, R.L.: The Inhalation Toxicity of the Refrigerant, Chlorodifluoromethane (F-22). Intntl. Cong. Toxicol., March 30-April 2, 1977, Toronto, Ontario, Canada. (Abstract).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30043-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Evaluation of Liver Function in vivo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBCB NIEHS
OTHER:	J. M. Patel	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ↓

A substantial amount of work has been done in the past by others in an effort to establish a useful in vivo liver function test in rabbits based on the elimination kinetics of bromsulfophthalein (BSP) and indocyanine green (ICG). We have observed that, consistent with the work of others, these dye tests are relatively insensitive indicators of chemical injury. On the other hand, it has been reported that there is no enterohepatic circulation of ICG in the rabbit. We have found this not to be so.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbits are lightly anesthetized in order to perform jugular venous cutdowns. Relatively large intravenous catheters are placed in other to be able to obtain accurately timed blood samples. ICG is injected intravenously, blood samples taken, and the serum concentrations of ICG determined in a spectrophotometer. All concentration response characteristics of the rabbit model and the analytical instrument are determined. Serum concentration profiles are characterized with respect to the following variables: dose of ICG, patency or occlusion of the common bile duct and/or the portal vein, and chemical injury by carbon tetrachloride.

MAJOR FINDINGS AND PROPOSED COURSE: Following the intravenous injection of ICG, there is a rapid rise in serum ICG levels through the first minute. The serum levels decline in a log-linear manner throughout the interval from 1 to 4-5 minutes post injection, following which the rate of decline decreases. Between 10 and 15 minutes post injection, the serum level begins to rise reflecting enteric reabsorption of the ICG. This rise is eliminated by occlusion of the common bile duce. The half-time for serum elimination of ICG measured between 1 and 4 minutes post injection provides the most reliable index of impaired ICG elimination by the liver. The half-time for elimination of ICG over the period of 1 to 4 minutes post injection is unaffected by occlusion of the common bile duct but is reduced substantially by occlusion of the portal vein. Occlusion of the portal vein deprives the liver of 85 to 90% of its circulation, thus reducing the delivery of ICG to the excretory organ. The half-time for elimination at 1 to 4 minutes is greatly decreased by occlusion of the portal vein.

The present method is invasive. Therefore, we propose to evaluate a non-invasive method using an ICG dye tracer that is attached to the pinna of the ear. The non-invasive dye tracer approach provides a continuous output in contrast to the present method that provides discrete samples. We propose to compare the dye tracer output using methods of wave form analysis to determine if this approach increases the sensitivity of the test.

Other dyes may lend themselves to this application also. We propose to evaluate fluorescein in this connection. Blood fluorescein levels are easily quantified and the compound is readily excreted by the liver.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Exposure to many environmental chemicals is known to impair liver function. Assessment of the impact of such environmental chemicals is dependent on appropriate testing procedures. We are, therefore, interested in exploring old and new methods of evaluating liver function, particularly non-invasive methods that have a potential for safely and economically screening segments of the population at risk.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30044-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Renal Toxicology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBCB NIEHS
	E. G. Tombropoulos	Research Chemist	EBCB NIEHS
OTHER:	M. P. Moorman	Biomedical Engineer	EBCB NIEHS
	A. G. E. Wilson	Visiting Associate	LP NIEHS

COOPERATING UNITS (if any)

Pharmacokinetics Section, LP, NIEHS

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Morpholine has been selected as the first chemical for evaluation. Morpholine is a cheap and widely used solvent. It is a heterocyclic secondary amine that would be expected to be excreted in the urine by way of the tubular organic base excretory mechanism. One of the apparent reasons why this compound has not been studied in the past is the difficulty of the analysis in biological fluids. A considerable amount of time has been devoted to establishing this methodology and we now have a reliable gas chromatographic method for its determination in blood and urine. A rabbit nose exposure unit has been assembled to include automatic GC monitoring. The pharmacokinetics and renal handling of morpholine are being studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbits are anesthetized with ketamine-xylazine. A plastic intravenous catheter is advanced to the level of the post cava by way of the right jugular vein. A catheter is implanted in the urinary bladder by a transabdominal approach. Animals are exposed to controlled concentrations of morpholine in a nose exposure unit. Blood and urine concentrations of morpholine are determined by gas chromatography. p-Aminohippurate (PAH), inulin, and tetraethyl ammonium (TEA) clearances are determined using radioactively labelled compounds. Other measurements made in connection with this study are performed on a blood gas analyzer, a vapor pressure osmometer, and a glucose analyzer using immobilized glucose oxidase.

MAJOR FINDINGS AND PROPOSED COURSE: This is a new study and we have no major findings at this time. Work to date has consisted of developing the method for the determination of morpholine in biological fluids and assembling, calibrating and operating a nose exposure unit with automatic GC monitoring.

We propose to evaluate the pulmonary uptake and elimination kinetics of morpholine in the rabbit. In addition, we will characterize the renal excretion of the compound in terms of glomerular filtration and tubular mechanisms.

We are collaborating with the Pharmacokinetics Section of the Laboratory of Pharmacology in studies of the comparison of the pulmonary uptake of morpholine *in vivo* versus the isolated perfused lung. These studies may be extended to include pulmonary elimination following exposure by either the pulmonary route, by ingestion, or *via* the pulmonary artery.

The molecular structure of morpholine suggests that it should be susceptible to biotransformation. We are prepared to consider this in further detail if preliminary observations suggest that a substantial amount of morpholine metabolism occurs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pulmonary and cardiovascular systems are tightly integrated with renal function. The assessment of the impact on the organism of exposure to environmental contaminants is dependent on a comprehensive understanding of the effects of impairment of interactive physiological functions. The role of the kidneys as regulators of cardiovascular function requires the scientific investigation of chemicals for which the target organ may be the kidney.

When indicated and practicable, our general approach to studies of the inhalation toxicology of gases will include closely integrated studies of pulmonary, cardiovascular, and renal function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30045-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of an Automatic Small Animal Inhalation Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. P. Moorman	Biomedical Engineer	EBCB NIEHS
	E. W. Van Stee	Physiologist	EBCB NIEHS
OTHER:	R. Dennis	Machinist	RSB NIEHS
	T. Orr	Machinist	RSB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves the design and implementation of an automatic data processing system to monitor and control gas concentrations in a small animal inhalation exposure facility. Included in this effort are the development of hardware, software and operational procedures necessary for the complete utilization of the system.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Data acquisition and feedback control theory have been used to develop an electronic calculator based sampled data control system capable of regulating 9 chambers on a time multiplex basis.

MAJOR FINDINGS AND PROPOSED COURSE: Since this system must regulate gases generated from compounds with different physical properties, it has been necessary to design a control system capable of measuring certain characteristics of each generating system and adapting the control equations to optimize responses for each particular compound.

The realization of full performance from this system requires further improvement in the adaptive control functions and the addition of operation and status monitoring equipment to provide the man-machine interface.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
1. Machine control of inhalation chambers can produce an accurately controlled and documented exposure resulting in reduced technical error.

2. Documentation of the exposure conditions and profiles can be summarized in a compact and meaningful format eliminating the need for hand analysis of chamber output data.

3. More complicated exposures such as time varying concentrations of multiple compounds are possible with machine control more than human operators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30046-01 EBCB

PERIOD COVERED

July 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Early Detection of Lung Injury

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Tombropoulos	Research Chemist	EBCB NIEHS
	E. W. Van Stee	Physiologist	EBCB NIEHS
OTHER:	W. Gibson	Chemist	EBCB NIEHS
	H. Lam	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pulmonary lavage is easily obtainable and can be taken at various time intervals from the same animal (large) without killing the animal. Pulmonary lavage contains lipoprotein surfactant, lung cells, macrophages, enzymes released from macrophages, and enzymes associated either with the protein part or the lipid part of the lung surfactant. Lung surfactants are important for the stability of alveoli; lipids not associated with lung surfactant may be related to lung cell membranes. Lung tissue is capable of synthesizing lung surfactant and lipids. The alveolar macrophages are vital entities of the mammalian immune system. In the latter capacity, the macrophage depends upon plasma membrane activity for migration recognition, activation, phagocytosis and attachment to substrate tissue.

Thus, lavage can be used as a probe for early detection of lung cell damage, and of the lung immune and biosynthetic function.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Animals will be exposed to gases and fibers that may be found as environmental contaminants. Exposures are carried out in dynamic flow through chambers of two types. One type provides whole body exposure and the other is a nose exposure unit.

Pulmonary lavages are obtained and the cellular, lipoprotein and lipid components are isolated by differential centrifugation. The ^{125}I-Wheat Germ Agglutinin binding to alveolar macrophages will be used as a probe of alteration in alveolar macrophage membrane structure. The lipids (those associated with surfactants and those that are free) will be separated into classes by T.L.C. and column chromatography and their fatty acid composition determined by G.L.C.

MAJOR FINDINGS AND PROPOSED COURSE: This is a new study. Activities to this date have been devoted to assembling the necessary equipment, organizing the laboratories, and establishing basic methodology.

We propose to begin this project with a study of two types of gaseous materials: (1) nonrespiratory irritants such as organic solvent vapors, and (2) respiratory irritants such as nitrogen dioxide. As the project gets underway we hope to extend these studies to particulates. We have an interest in respirable glass fibers and asbestos.

Some asbestos preparations are known to be cytolytic because of the relatively high ratio of magnesium to silica at the surface of the fibers. The amount of magnesium on the surface can be reduced by prewashing the fibers with oxalate solutions. Changes of the magnesium to silica ratio on the surfaces of asbestos fibers greatly reduces its cytolytic effect. A preliminary study will involve exposure of groups of rats to oxalate washed and unwashed respirable asbestos particles. Pulmonary lavages will be examined for evidence of early lung injury vis-a-vis the presence of the surface magnesium.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A great need exists for the development of indices of early toxic injury to the lung. This is particularly true for lung carcinogens, i.e. initial, relatively brief, exposures of humans to asbestos is followed by latent period of 15 to 20 years before tumorogenesis becomes detectable. Virtually nothing is known about possible initial toxic responses of the nature of the processes leading to tumorogenesis during the long latent period. Furthermore, wo do not know it there is a connection between a toxic injury and carcinogenesis.

The lung is a central organ in matters related to inhalation toxicology. The lung serves the function of portal of entry, site of biotranformation, and an organ of elimination for many compounds. Soluble compounds to which the organism is exposed by inhalation may or may not have a deleterious effect on the lung itself. The lung may constitute the organ through which

the parent compound and/or its products of biotransformation are introduced into the organism with the result of the production of tissue injury to the lung itself or other organs of the body. The ojective of this project is the search or evidence of early toxic injury that may shed light on subsequent events.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30047-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanisms of the Dehalogenation of Low Molecular Weight Halogenated Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBCB NIEHS
	E. G. Tombropoulos	Research Chemist	EBCB NIEHS
OTHER:	W. Gibson	Chemist	EBCB NIEHS
	H. Lam	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Toxic injury as the result of exposure to low molecular weight halogenated compounds is often the result of the formation of pharmacologically and/or toxicologically active products of biotransformation. An understanding of the mechanisms of toxic chemical action requires the elucidation of metabolic pathways of the compounds in question. Vinyl bromide has been known for some time to be decomposed in vivo with the liberation of inorganic bromide and other unknown products. We have demonstrated that exposure of rats to hepatotoxic concentrations of vinyl bromide by inhalation results in the liberation of pharmacologically significant quantities of inorganic bromide. Serum inorganic bromide levels rose to levels high enough to cause a quantifiable central nervous system depression. The affects of bromide on the kidney in this context, the principle organ of excretion of bromide, remains unknown. Likewise the identity and fate of other products of biotransformation remain unknown.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Only *in vivo* studies have been performed to date. Rats have been exposed by inhalation in dynamic flow through chambers to vinyl bromide. Serum inorganic bromide levels were determined by standard chemical means. CNS depression was quantified by measuring the prolongation of the duration of hexabarbital induced sleep.

In vivo studies of defluorination have been carried out in rabbits exposed to $CHClF_2$ in dynamic flow through chambers and mice exposed to the various fluorinated alkanes and ethers in small static chambers. Bone levels of inorganic fluoride were determined in EDTA elluents by means of a fluoride specific electrode. The accumulation of inorganic fluoride in the bone serves as an index of defluorination. This is particularly useful when defluorination is known or thought to proceed to a very limited extent.

9,000 x g and 105,000 x g microsomal preparations and soluble enzyme systems derived from various organs, particularly the liver, will be used to study dehalogenation mechanisms *in vitro*. Particular attention will be paid to the mixed function oxidases associated with the smooth endoplasmic reticulum, glutathione, and glutathione-S-transferase systems. The effects of anaerobiosis on dehalogenation pathways will be studied.

MAJOR FINDINGS AND PROPOSED COURSE: Serum levels of inorganic bromide in rats were proportional to the duration of inhalation exposure to 6% vinyl bromide in air. Central nervous system depression measured as a function of the prolongation of the duration of hexobarbital induced sleep was proportional to serum bromide levels. Other products of the biotransformation of vinyl bromide remain unidentified. The evidence does, however, support the contention that these products are not toxicologically significant central nervous system depressants.

Slight and statistically insignificant increases in bone inorganic fluoride were detected after prolonged exposure of rabbits to $CHClF_2$. A definitive answer to the question of whether or not defluorination of the compound takes place and, if so, does it proceed to a toxicologically significant extent, must await further studies. Because it requires many weeks of daily exposure of rabbits to $CHClF_2$ before any toxic response becomes manifest, we have completed these studies on only a relatively small number of animals. Future studies are expected to include rats and mice as well.

We have begun a validation of the bone fluoride technique in our laboratory as an index of defluorination *in vivo*. Groups of mice are placed on distilled water as controls, distilled water containing potassium bromide, or exposed by inhalation to methoxyflurane, an inhalation anesthetic known to be readily defluorinated in such model systems. Following the successful validation of this technique we propose to extent these studies to include a wide variety of related fluorinated compounds.

In vitro studies will follow the acquisition of preliminary data from the in vivo experiments. They will be conducted according to the methods cited above.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A complete understanding of the mechanism of toxic action of low molecular weight halogenated compounds is predicated not only on the uptake, distribution, and histopathological manifestations of toxicity, but also the metabolic fate of the compound as well. Often the proximate toxicant is a product of the biotransformation of the compound rather than the parent compound itself. Furthermore, biotransformation of the parent compound in one organ may give rise to a product of biotransformation which is secondarily distributed throughout the body to become a proximate toxicant in another organ. Finally the distribution and retention of the compound throughout the body as well as its elimination is always markedly affected by its metabolism.

PUBLICATIONS

Van Stee, E.W., Patel, J.M., Gupta, B.N., Drew, R.T.: Consequences of Vinyl Bromide Debromination in the Rat. Sixteenth Annual Meeting, Society of Toxicology, Toronto, Canada, March 27-30, 1977. (Abstract)

Van Stee, E.W., McConnell, E.E., Patel, J.M., and Hamlin, R.L.: The Inhalation Toxicology of the Refrigerant, Chlorodifluoromethane (F-22). International Congress on Toxicology, Toronto, Canada, March 30-April 2, 1977. (Abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30048-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Halogenated Biphenyls and Napthalenes on Hepatic Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Goldstein	Pharmacologist	EBCB NIEHS
OTHER:	P. C. Linko	Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

J. D. McKinney, J. R. Hass, L. Levy, Chemistry Section, EBCB

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ⊦

The purpose of this study is to evaluate halogenated biphenyls and napthalenes with regard to: (1) classification of the administered agent as a dibenzo-dioxin or phenobarbital inducer; (2) compare the dose-response of induction of various isomers; (3) relate potency to tissue levels and toxicity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats were given single or multiple graded doses of Firemaster BP-6 (FF-1), 2,4,5,2',4',5'-hexabromobiphenyl (HBB) or 2,3,6,7-tetrabromonaphalene both orally and i.p. to evaluate the effects and potency of these compounds on various liver enzymes.

MAJOR FINDINGS AND PROPOSED COURSE: 2,4,5,2',4',5'-HCB was found to be much less potent than the commercial mixture of FF-1, and to resemble phenobarbital in its effects. FF-1 had effects intermediate between those of phenobarbital and the chlorinated dibenzodioxins. It shifted the peak of P-450, altered the ethyl isocyanide difference spectrum and induced aryl hydrocarbon markedly while 2,4,5,2',4',5'-HBB had none of these effects. It was also more potent as an inducer than HBB. Hepatic recovery from FF-1 was slight in a 30-day recovery study. This project is to be continued. Dose response curves for acute exposure to HBB, FF-1 and tetrabromonapthalene are underway.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dose response curves for chlorinated biphenyls, and their contaminants, the chlorinated dibenzofurans have indicated that the hepatic effects of PCBs can be predictive of their general toxicity. Those compounds, which like the polycyclic hydrocarbons, increase AHH, and alter the spectral characteristics of P-450 are the most toxic and the most potent. This system allows a quantitative measure of the relative hepatic effects of the various halogenated compounds of these complex mixtures.

PUBLICATIONS

Goldstein, J.A., Hickman, P., Bergman, H., McKinney, J.D., and Walker, M.P.: Separation of Polychlorinated Biphenyl (PCB) Isomers into Two Distinct Types of Inducers of Drug-Metabolizing Enzymes. Fed. Proc. 36: 941, 1977.

Goldstein, J.A., Hickman, P., Bergman, J., McKinney, J.D. and Walker, M.P.: Separation of Pure Polychlorinated Biphenyl Isomers into Two Types of Inducers on the Basis of Induction of Cytochrome P-450 or P-448. Chem.-Biol. Interact. 17: 69-87, 1977.

Goldstein, J.A., Hass, J.R., Hickman, P., and Harvan, D.J.: Chlorinated dibenzofurans and Dibenzo-p-dioxins in a Commercially Available 99% Pure Polychlorinated Biphenyl Isomer Identified as the Inducers of Hepatic Cytochrome P-448 and Aryl Hydrocarbon Hydroxylase in the rat. Drug Metab. Disp. (Submitted)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30049-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Identification and Characterization of Environmental Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Staff Fellow	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	R. H. Cox	Visiting Scientist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Negative chemical and electron impact ionization mass spectrometry is being used for quantitative analysis of chlorodibenzo-p-dioxins in dairy cow tissue extracts. These dioxins were also found in the pentachlorophenol treated lumber from the barns used to house these animals.

The flame retardant Firemaster BP-6 has been subjected to chemical and toxicologic study. No evidence could be found for the presence of bromodibenzo-p-dioxins or bromodibenzofurans. The material analyzed was found to be contaminated with a few hundred ppm bromonaphthalenes

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard GC/MS techniques, negative chemical ionization mass spectrometry, high pressure liquid chromatography, rabbit ear bioassay, histological examination.

MAJOR FINDINGS AND PROPOSED COURSE: A dairy herd from Michigan was found to be contaminated with chlorodibenzo-p-dioxins. Liver samples contained between 1 and 150 ppb octa-, <.01 and 20 ppb hepta- and <.01 to 1 ppb hexachlorodioxin. Somewhat lower levels were found in the fat. We propose to analyze these samples for other likely environmental contaminants and to analyze samples from another farm in a different locality.

The polar fraction of Firemaster BP-6 was subjected to mass spectrometric analysis, chemical degradation followed by GC/MS analysis of the products and toxicologic evaluation. No evidence could be found to support the presence of bromodibenzo-p-dioxins or bromodibenzofurans or other highly toxic impurities in this fraction. The non-polar fraction was found to have toxic properties similar to those of the crude material. The crude Firemaster was found to be contaminated with a total of approximately 200 ppm tetra and pentabromonaphthalenes. We propose to separate the non-polar fraction of Firemaster by means of high pressure liquid chromatography and identify the bromobiphenyls present.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A precise understanding of the chemical nature of environmental hazards is needed to select the most appropriate chemicals for biological studies.

PUBLICATIONS

Hass, J.R., McConnell, E.E., and Harvan, D.J.: Chemical and Toxicologic Evaluation of Firemaster BP-6. J. Food. Agric. Chem. (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30050-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemical Conjugation of Specific ^{13}C-Labeled Benzo[a]pyrene Oxides to Glutathione: Regiospecificity and Stereospecificity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB	NIEHS
OTHER:	R. H. Cox	Visiting Scientist	EBCB	NIEHS
	O. Hernandez	Visiting Fellow	LP	NIEHS
	B. Smith	Staff Fellow	LP	NIEHS
	J. Bend	Visiting Scientist	LP	NIEHS

COOPERATING UNITS (if any)

Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. NO1-CP-33387. Supplemented by NIEHS).

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the chemical conjugation of specific ^{13}C-labeled benzo[a]pyrene oxides to glutathione, enzymatically and non-enzymatically. The regiospecificity and stereospecificity of the reaction will be determined. Mechanistic elaboration of the binding process is important in understanding, preventing, and predicting the mechanism(s) of action of such compounds. Several specifically ^{13}C-labeled benzo[a]pyrene derivatives have been obtained and studies of their chemical and biological conjugation have been initiated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy; organic syntheses, in vitro biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: Several benzo[a]pyrene derivatives of high purity and definition including two oxides labeled at specific positions and known enrichments with non-radioactive ^{13}C have been obtained. Studies with benzo[a]pyrene-4,5-oxide-4,5-$^{13}C_2$ have been initiated to determine the preferred position of attack on the arene oxide by glutathione and the stereospecificity of the addition product. The detoxifying glutathione S-transferase enzyme system previously shown to covalently bind certain epoxides will be used. Future studies should include competition experiments to determine which biological nucleophiles are most reactive and the enzymatic versus non-enzymatic nature of the ring opening process and, if possible, elucidation of the required microenvironment for the covalent binding of such arene oxides. ^{13}C-NMR spectra of the various oxygenated derivatives will be recorded routinely for reference purposes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Certain arene oxides are known to bind covalently to nucleic acids, and they have been implicated as carcinogens and mutagens which can be derived from the parent compounds. The abundant detoxifying glutathione S-transferase enzyme system is also likely to covalently bind these oxides. Such mechanistic elaboration of the process will aid in understanding, preventing, and predicting the mechanism(s) of action of these compounds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30051-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Characterization of Specific Binding (Electronic) Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
OTHER:	R. H. Cox	Visiting Scientist	EBCB NIEHS
	P. W. Albro	Research Chemist	EBCB NIEHS
	K. K. Kohli	Visiting Fellow	EBCB NIEHS
	J. A. Goldstein	Pharmacologist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the specific binding character of certain toxic polyhalogenated aromatic compounds using purified biopolymers, their monomers and other isolated and definable biological systems as models. An attempt is being made to correlate binding propensity with certain kinds of biological activity. Early work has shown quantitative differences in binding for symmetrical hexachlorobiphenyl isomers.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy using specifically labeled (^{13}C, ^{19}F, and ^{2}H, etc.) compounds where possible for studying complex molecular interactions. Isolation and characterization of specific binding site(s) in body tissue and fluid using standard biochemical methods.

MAJOR FINDINGS AND PROPOSED COURSE: There is an increasing body of evidence that the chlorinated dibenzodioxins and dibenzofurans, and certain chlorinated biphenyls have a similar biological mechanism of action which may be initiated through specific binding of the parent compound. Studies to date have established that one of the mechanisms whereby a polychlorinated biphenyl becomes bound to biological systems is through the formation of a charge-transfer complex. This finding implicates aromatic amino acids and the purine bases as possible binding sites for the polychlorinated biphenyls. Investigations with 2,4,6,2',4',6'- and 3,4,5,3',4',5'-hexachlorobiphenyl show that 3,4,5,3',4',5'-hexachlorobiphenyl is the more tightly bound of the two isomers which is also the more toxic. This is thought to reflect the relative ease with which the two rings of the hexachlorobiphenyl can achieve a planar configuration in solution. Future studies should include an examination of the binding of other polychlorinated biphenyls, including those representing potential metabolites and steric and electronic probes, with biopolymers, their monomers as models and proceed to various intracellular organelles and their specific biochemical systems isolated from biological tissue and fluid. An attempt will be made to develop techniques to overcome problems of solubility associated with the presentation, of essentially water insoluble halogenated hydrocarbons to water soluble biological materials. Later work will extend this approach to include the toxic dibenzo-*p*-dioxins and dibenzofurans.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing evidence that certain highly toxic chlorinated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

Goldstein, J.A., Hickman, P., Bergman, H., McKinney, J.D., Walker, M.P.: Polychlorinated Biphenyl Isomers: The Influence of Position and Degree of Chlorination on Induction of Cytochrome P450, P448, and Drug Metabolizing Enzymes in the Liver. Chemical Biological Interactions. (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30052-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
^{13}C-Nuclear Magnetic Resonance Study of Chlorinated Polycyclodiene Pesticides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
OTHER:	R. H. Cox	Visiting Scientist	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{13}C-Nuclear Magnetic Resonance (NMR) is being used to characterize several chlorinated polycyclodiene pesticides and some of their phototransformation and potential metabolic products. ^{13}C-NMR was shown to be superior to proton NMR for identification of these compounds and elucidation of their peculiar electronic interactions which underlie their biological activity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C nuclear magnetic resonance (NMR) spectroscopy and shift and relaxation reagents.

MAJOR FINDINGS AND PROPOSED COURSE: The ^{13}C NMR spectra of several chlorinated polycyclodiene pesticides including aldrin, dieldrin, endrin, and heptachlor, and some of their phototransformation and potential metabolic products (derived from synthesis), have been obtained and interpreted. ^{13}C-NMR was shown to be superior to proton NMR for the identification of these compounds and will aid in elucidating the peculiar electronic interactions in these polycyclic systems which underlie their biological activity. This study will be made as comprehensive as possible within the limitations of our synthetic capabilities.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ultimate fate of the chlorinated polycyclodiene pesticides in the environment continues to be investigated. Therefore, more difinitive techniques to identify their various possible transformation products are needed. Furthermore, it is likely that the biological activity associated with many of these compounds is a function of their peculiar electronic interactions, intra- and intermolecularly.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30053-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Polybrominated Biphenyls on Immunocompetence in Cattle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. I. Luster	Staff Fellow	EBCB NIEHS
	R. E. Faith	Senior Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.16	0.08	0.08

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies were undertaken to investigate the effects of in utero exposure to polybrominated biphenyls (PBB) on immunocompetence in cattle. Exposure to PBB occurred through contaminated feed accidentally shipped to two Farm Bureau Feedmills in Michigan in 1973. Cattle in this immune study consisted of 17 calves exposed in utero and 13 aged matched controls maintained at Purdue University, Indiana. Since this was a field study, only a limited immunological work-up could be performed. However, these studies indicated that a state of hyperimmunity presently exists in the exposed cattle.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated (T-cell) immunity was assessed *in vitro* by the response of peripheral blood lymphocyte cultures to the mitogens Concanavalin A and phytohemagglutinin. Humor immunity (B-cell mediated) was assessed by radial immunodiffusion to determine serum IgG, IgM, and IgA levels, hemagglutination and conglutinin assays to determine natural antibody titers to SRBC and the response of pheripheral blood lymphocyte cultures to poke weed, a B- and T-cell mitogen.

MAJOR FINDINGS AND PROPOSED COURSE: Results of these studies have indicated that *in utero* exposure to PBB in cattle, induces at this particular time following exposure, a state of hyperimmunity especially with regards to cell mediated immunity. This was evidenced by a significant increase in mitogenic stimulation. While most humoral immune functions appeared normal, a significant increase in serum IgA levels occurred in exposed animals. It is interesting to note that in contrast to the other classes of immunoglobulins, IgA is under control of the thymus.

An intramural study is underway to determine the dose related spectrum of toxicologic effects associated with ingestion of PBB in rats and mice. The immunologic profile of these animals will be determined.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Ingestion of polybrominated biphenyls appears to exert toxicologic effects in man and animals. Among these effects may be a modulation of the immune response, either suppression or enhancement. It is important to establish the nature and mode of action of this immune modulation in order to evaluate the possible hazards for man and animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30054-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Passive Immunization in TCDD Exposed Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. I. Luster	Staff Fellow	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	P. W. Albro	Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.07	0.05	0.02

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

C57Bl/6 mice were passively immunized with antibodies to 2,3,7-trichloro-dibenzo-p-dioxin (TriCl-DD) one day prior or one day following treatment with 250 mg/Kg (LD_{50-30}) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an attempt to explore the protective capabilities of passive immunization against dioxin exposure. Statistical analysis of body weight loss and mortality indicated no protective effect of passive immunization to TCDD exposure.

PROJECT DESCRIPTION

METHODS EMPLOYED: Gamma-globulins from normal and immune rabbits were prepared by passage over DEAE-SEPHADEX columns. The antibody binding capacity to TriClDD was determined by immunodiffusion analysis in gels and the $(NH_4)_2\ SO_4$ radioimmunoassay (Farr Assay). The γ-globulins were prepared for injection by extensive dialysis with phosphate buffered saline and concentration by ultrafiltration. Exactly 0.4 ml of the preparation (equivalent to 6 ml whole serum) was administered I.M. in the thigh. TCDD (250 mg/Kg body weight) was administered orally. Mice were divided into 5 groups and injected with either normal rabbit γ-globulin (NGG), NGG and TCDD, immune γ-globulin one day prior to TCDD exposure, immune γ-globulin one day following TCDD exposure or TCDD alone.

MAJOR FINDINGS AND PROPOSED COURSE: These studies suggest that passive immunization would not be of value in treatment for TCDD exposure. Attempts at cell mediated passive transfer (transfer factor) as well as active immunization are being considered.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful treatment for TCDD exposure in man and animals is evidently lacking. Any positive therapy has obvious importance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30055-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanism of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Toxicity in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
OTHER:	P. W. Albro	Research Chemist	EBCB NIEHS
	H. B. Matthews	Research Chemist	LP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to investigate the mechanism of toxicity and cause of death in rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Specifically, the affect of TCDD on fat absorption, metabolism and transport from the small intestine.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer 344 rats were given a single oral LD_{100} (80 µg/kg) of TCDD in corn oil. At various times thereafter, they were given 1 ml of olive oil. At 2, 4 and 6 hr after olive oil, portions of small intestine were taken for light and electron microscopy. Subsequent quantitative studies used C^{14} labeled triolin and C^{14} oleic acid.

MAJOR FINDINGS AND PROPOSED COURSE: As early as 3 days post TCDD exposure, there was a reduction in the rate of fat absorption from the small intestine. That absorbed remained in the intestinal epithelial cell longer than normal. In addition, ultrastructural analysis of the cell indicated that the fat globules are much larger than normal and are poorly transferred out of the cell. Data on the C^{14} labeled aspects of the study are incomplete. This project will be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TCDD is a highly toxic chemical that may be found as an unwanted contaminant of useful compounds. The lesions of toxicity have been described in several animal species, both in acute and chronic dosing regimens. However, in those cases where death occurs, it is difficult to explain the organopathologic basis. Most animals appear to waste away in a starvation-like manner. Halogenated biphenyl and dibenzofuran toxicity produces a similar syndrome. It would be extremely useful to be able to explain the mode of death, in the hope that preventative therapy could then be developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30056-01 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparison of NIH Feed 34 to NIH Feed A as a Guinea Pig Ration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Senior Staff Fellow	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was undertaken to evaluate NIH feed 34 as an autoclavable guinea pig ration. Guinea pigs fed NIH feed 34 had body weight gains and feed consumption equal to animals fed the standard guinea pig ration, NIH feed A.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Forty weanling guinea pigs were randomly divided into 2 groups of 20. One group was fed autoclaved NIH feed 34 for 60 days, the other group was fed unautoclaved NIH feed A. Body weight gains and feed consumption was recorded for both groups.

MAJOR FINDINGS AND PROPOSED COURSE: Guinea pigs fed NIH feed A or NIH feed 34 gained body weight and consumed feed at the same rates. At necropsy, there was no observable difference between the two groups. Hematological parameters were the same in both groups.

It is proposed that NIH feed 34 be evaluated as a rabbit ration and then these studies be terminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: When NIEHS occupies the permanent facility, the animal facility will be run as a barrier colony. Animal feed entering the animal colony will be distributed from a central distribution system. All feed entering this distribution system will be autoclaved prior to entering the system; therefore, it is necessary to utilize an autoclavable guinea pig ration in the permanent facility.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30057-01 EBCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Evaluation of Toxicologic Effects of Pentachlorophenol and its Contaminants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)

James D. McKinney, Ph.D., J. R. Hass, Ph.D., and M. I. Luster, Ph.D., Chemistry Section, EBCB; Dept. of Animal Husbandry, N. C. State University

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evaluation of tissues collected from dairy cattle in a barn recently constructed with pentachlorophenol treated lumber detected the presence of various chlorinated dibenzodioxins. The presence of these contaminants of pentachlorophenol in animal tissues suggests that studies be undertaken that define the circumstances associated with body accumulation of these contaminants as a consequence of exposure to pentachlorophenol. Attempts should be made to simultaneously correlate any adverse health effects that may be associated with body burdens of these chemical contaminants.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Through the feeding to cattle of varying doses of pentachlorophenol that have been characterized as to the nature and quantity of their dibenzodioxin and dibenzofuran contaminants, determine the types and quantities of contaminants that may accumulate during chronic exposure (120 days). Selected tissues will be analyzed by appropriate gas chromatographic/mass spectrometric methods. Using standard toxicology methods, evaluate clinical effects that may be associated with the feeding of pentachlorophenol and its contaminants as well as perform a complete gross and microscopic examination of selected tissues. Determine the effects of administration of these chemicals on immune function employing standard in vivo and in vitro immunologic techniques such as response to known antigens, lymphocyte response to mitogen stimulation, mixed lymphocyte cultures, etc.

MAJOR FINDINGS AND PROPOSED COURSE: This study has commenced. Dependent upon results of this initial experiment, a subsequent study may be performed that assesses distribution and secretion of these contaminants into the fetus and milk, respectively.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The dibenzodioxins and dibenzofurans are some of the most toxic man made chemicals known to man. Their ability to accumulate in tissues or milk products destined for human consumption need to be assessed as to possible public health hazard. These studies will allow us to further extend our research into the toxic properties of these classes of chemicals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80017-06 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Inhalation Toxicity of Compounds Common to Household Aerosols

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
OTHER:	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	J. Takezawa	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

Histology Section, EBCB
Environmental Biometry Branch

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inhalation toxicity of commercial aerosol products and separate components of aerosol products are being studied. Animals are exposed to the compounds under dynamic conditions and the toxicity is evaluated using several indices, including organ weights, morphological changes, changes in hepatic and pulmonary enzyme activity and respiratory physiology. Compounds of current interest include aluminum-chloride-hydroxide, oven cleaners, spray starches and fluorocarbon propellants.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Exposure Techniques: Exposures are carried out under dynamic conditions with contaminant concentrations being monitored frequently during exposures. Aerosols of aluminum-chloride-hydroxide are generated from an aqueous solution with a compressed air-driven nebulizer. Commercial aerosols are ejected into an elutriation chamber which traps the larger particles and then into an exposure chamber. The cans are activated mechanically on a 5-second on - 25-second off cycle. The fluorocarbons are blended with compressed air to the concentration desired and then directed to the chamber or in some cases to an endotracheal tube for direct exposure.

2. Toxicity Evaluation: Several techniques are utilized to determine the effects of exposures. These include histologic preparation of tissue and microscopic examination for pathological or morphological changes; investigation of mixed-function oxidase (MFO) activities of both lung and liver microsomes after animal exposure; assessment of weight gain and organ weights after exposure; investigation of any change in respiratory physiological parameters such as breathing rate and volume, resistance, and static and dynamic compliance; measurement of electrocardiographic and hemodynamic parameters, and myocardial contractility changes after and during exposures; and measurement of deposition and clearance of chemicals in the lung, and body distribution and elimination of chemicals after exposure.

MAJOR FINDINGS AND PROPOSED COURSE: Total lung capacity was significantly reduced in animals exposed to 50 mg/m^3 for 6 hr/day, 5 days/wk for one month. Diffusing capacity of the lung for carbon monoxide was only slightly reduced. This is consistent with the histological findings which showed little or no involvement of parenchymal tissue and a significant accumulation of cells in the air spaces of the lungs.

The project is terminated with this report.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of the aerosol "spray can" dispenser has resulted in a very complex and wide variety of materials being available in respirable form. While the acute toxicity of many of the "active ingredients" in such dispensers has been investigated, little is known about the chronic toxicity of these compounds or the toxicity of combinations of these compounds and "inert ingredients" of the pressurized cans. Elucidation of the chronic toxicity of these compounds and their potential combinations can be of vital importance to environmental health.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80018-07 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Indices of Inhalation Exposure to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	F. Englebrecht	Visiting Scientist	EBCB NIEHS
OTHER:	J. Patel	Visiting Fellow	EBCB NIEHS
	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	E. W. Van Stee	Physiologist	EBCB NIEHS
	R. M. Philpot	Pharmacologist	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a). HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this long term project the effects of inhaled solvents are being investigated. Biochemical indices of pulmonary and hepatic injury are being studied using serum enzymes as well as pulmonary and hepatic microsomal enzymes. Oxygen consumption of lung slices is being used to assess toxicity of these agents. Previous compounds investigated include benzene and dichloromethane. Agents presently being studied include vinyl bromide and paraquat.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Inhalation exposures are carried out in chambers specifically designed for such studies. They are operated under dynamic conditions of air flow and atmospheric concentrations of specific compounds are monitored at frequent intervals. Biochemical assays are carried out in routine fashion.

We have adapted methods to estimate metabolism for use with pulmonary tissue and with subcellular fractions. Patterns of substrate utilization, oxygen consumption, and enzyme activities have been used to quantify metabolism in whole lung, lung slices, homogenates and isolated lung mitochondria.

MAJOR FINDINGS AND PROPOSED COURSE: Changes in oxygen consumption, substrate utilization (e.g. glucose consumption, lactate production, and substrate oxidation) and enzyme activities of lung tissue exposed to toxicants such as paraquat and oxygen have been measured. Marked metabolic changes occur after exposure to such toxicants and these changes are evident after acute and chronic exposure. Additional information regarding normal lung physiology and the response to a number of types of compounds needs to be gathered.

Intermittent daily exposure of rats to 2% VB caused weight loss during the first few days of exposure. The weight loss was increased by the inclusion of phenobarbital in the drinking water. Results of paired feeding studies suggested that most of the weight loss could be attributed to decreased food intake. The reason for the decreased food intake was determined to be the consequence of a combination of anesthetization by the VB plus an additive increment of CNS depression associated with an elevated serum bromide concentration. The purely bromide components were quantitatively similar whether the bromide was derived from the debromination of VB or from inorganic bromide included in the drinking water. The phenobarbital effect was attributed to induced acceleration of VB debromination with the subsequent liberation of increased amounts of bromide.

Toxic injury to the liver observed during the first 2 days of exposure resolved by the 5th day of exposure. An additional weight loss in the VB plus phenobarbital group, not attributable to reduced food intake, corresponded to the period during which hepatotoxicity was histopathologically definable. Recovery of weight gains paralleled recovery from hepatotoxic injury.

We propose to elucidate further the metabolic fate of vinyl bromide through a combination of *in vivo* and *in vitro* testing. Although the toxicological effects of the inorganic bromide have been established, nothing is known of other products of biotransformation and their effect on the organism. The hepatic lesion would be expected to be caused by a reactive intermediate in the metabolic breakdown of vinyl bromide.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In today's society the number of people taking drugs either prescribed by a physician or illicitly is substantial. Some of these compounds stimulate liver microsomal enzymes, and possibly lung systems, thus increasing the rate at which certain chemicals and/or other endogenous substrates are metabolized. These studies are designed to better define the toxic responses to selected solvents and to determine if the toxicity of these solvents can be altered by pre-exposure to themselves or to other drugs. The effect of the route of administration is also being studied.

Vinyl bromide was selected because of its similarity to vinyl chloride and because of its use as an intermediate in the manufacture of fire retardants.

Surprisingly little is known regarding pulmonary intermediary metabolism and changes which might occur in response to insult, in spite of the fact that the lung is the first organ exposed to airborne pollutants. It is apparent, that in addition to gas exchange, the lung plays a vital role in the removal, metabolism and release of substances in the blood as well as the synthesis and secretion of substances peculiar to the lung. An understanding of the intermediary metabolism of pulmonary tissue may contribute to our understanding of normal lung function and function and to changes which occur early in response to toxicant exposure or disease.

PUBLICATIONS

Tierney, D.F., Young, S.L., O'Neil, J.J. and Abe, M.: Isolated Perfused Lung - Substrate Utilization. Fed. Proc. 36: 161-165, 1977.

O'Neil, J.J. and Engelbrecht, F. M.: Increased Oxygen Consumption and Pentose Pathway Activity of Rat Lung Slices by Paraquat in the Presence of Glucose. Fed. Proc. 36: 480, 1977. (Abstract)

Young, S.L., Kasuyama, R.S., O'Neil, J.J. and Tierney, D.F.: Lung Metabolism During Pulmonary Edema. Clin. Res. 25: 165, 1977. (Abstract)

O'Neil, J.J., Engelbrecht, F.M. and Wilson, A.G.E.: Paraquat Uptake and Distribution by Rat Lung. Am. Rev. Resp. Disease. (In press, 1977).

Van Stee, E.W., Patel, J.M., Gupta, B.N., Drew, R.T.: Consequences of Vinyl Bromide Debromination in the Rat. 16th Annual Meeting, Society of Toxicology, Toronto, Ontario, Canada, March 27-30, 1977. (Abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80020-05 EBCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
OTHER:	J. Takezawa	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Changes in pulmonary function resulting from inhalation of airborne pollutants are being estimated using a whole body pressure plethysmograph constructed for use with small animals. Both acute and chronic exposure to oxidants, singly and in combination with other compounds result in functional changes. Changes in pulmonary mechanics (e.g. resistance, compliance), spirometry (e.g. lung volumes, frequency of breathing), heart rate, and pulmonary morphology will be estimated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Tests have been adapted or devised for use in rodents to measure spirometry (lung volumes and capacities), mechanics (pulmonary resistance and compliance), ventilation (nitrogen washout), and the diffusing capacity of the lungs for carbon monoxide. For the most part these tests can be accomplished by using non-invasive techniques.

MAJOR FINDINGS AND PROPOSED COURSE: We found a positive relationship between the size of the experimental animal (50 g to 3000 g) and the magnitude of measurements such as lung volumes or diffusing capacity. In addition, these measurements of pulmonary function changed as animals aged (relative functional ability decreased). Chronic inhalation exposure to toxicants such as oxygen, NO_2, or aluminum chlorhydrate produced functional changes which correlated with the morphological and morphometric changes measured in animals with an identical exposure history. Such tests appear to be well suited to the assessment of function capabilities of laboratory animals and we propose to use them to estimate changes which occur following exposure to known or suspected pulmonary toxicants.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Changes in pulmonary function of animals after exposure to inhaled environmental agents are known, in many cases, to be associated with cellular damage and morphologic changes. Development of sensitive methods to detect pulmonary function changes may provide an early marker of lung damage and provide insight into both the mechanism of pulmonary damage and the types of location of the cells most readily injured by specific agents. Correlation of these findings with metabolic and morphologic changes may provide insight into the sequence of pulmonary damage.

RESEARCH RESOURCES PROGRAM CONTRACTS

IIT RESEARCH INSTITUTE - Chicago, Illinois 60616
(NIH-NO1-ES-6-2138)

TITLE: Radiosynthesis of ^{14}C-2,3,7,8-Tetrachlorodibenzofuran (TCDF)

CONTRACTOR'S PROJECT DIRECTOR: Allan P. Gray, Ph.D.

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research Chemist, Chemistry Section, EBCB

DATE CONTRACT INITIATED: June 25, 1976

CURRENT LEVEL (8 months): $14,695

PROJECT DESCRIPTION

OBJECTIVES: Synthesis of 10 millicuries of ^{14}C-radiolabeled 2,3,7,8-TCDF at the highest specific activity reasonably achievable but not less than 50 millicuries/millimole and with a chemical and radiochemical purity greater than 98 percent.

METHODS EMPLOYED: Usual radiosynthetic techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The compound, 2,3,7,8-TCDF has been previously synthesized unlabeled by IIT; however, its synthesis as a radiolabeled compound will require many different considerations and development of methodology. This contract has provided for an aborted attempt to prepare this compound labeled which has shown that the selection of which precursor compound should contain the label is critical in the radiosynthesis. This result was unexpected and appears to be related to the high specific activity and accompanying radiochemistry of the phenolic precursor compound used. Therefore, we propose to repeat the entire reaction scheme (critical step is diphenyl ether synthesis) incorporating the label in the other precursor compound (^{14}C-labeled bromobenzene).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated dibenzofurans occur as contaminants in commercially produced polychlorinated biphenyl (PCBs). Currently there is little known about the toxicity and biological fate of these compounds. It is presently assumed that the 2,3,7,8-TCDF is the most toxic member of the class of chemicals. Studies at NIEHS have shown it to be highly toxic for the guinea pig, but markedly less toxic for mice and rats. Studies on these species' difference in regard to variation in adsorption, metabolism, distribution and excretion are urgently needed. Such studies are most rapidly and conveniently done using radiolabeled compounds.

NATIONAL ACADEMY OF SCIENCES - Washington, D. C. 20418
(NIH-NO1-6-2128)

TITLE: "Partial Support for the Research of the Panel to Review Statistics on Skin Cancer"

CONTRACTOR'S PROJECT DIRECTOR: Margaret E. Martin, Ph.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D., Research Contracts Office, RRP

DATE CONTRACT INITIATED: April 15, 1976

CURRENT ANNUAL LEVEL: $26,400

PROJECT DESCRIPTION

OBJECTIVES: Further analysis and evaluation of statistics on skin cancer that are used to estimate the effect on the incidence of skin cancer of increased intensities of ultraviolet radiation that may result from reduction of stratospheric ozone.

METHODS EMPLOYED: Comparison will be made between the skin cancer surveys of the NCI and the M.D. Anderson Hospital and Tumor Institute; analysis will be completed on recent data from the NCHS Health and Nutrition Examination and detailed data on melanoma mortality; preparation of a revised estimate of projected percentage increase in skin cancers; and, suggestions for design of new experiments and manner of data collection on incidence and prevalence of skin cancer.

MAJOR FINDINGS AND PROPOSED COURSE: The panel has met to consider available preliminary data. Further meetings and analysis are planned on receipt of additional data from the National Center for Health Statistics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The report of the panel, when prepared, will provide information pertinent to a potential environmental health problem, i.e., the possibility of the increase in skin cancer due to environmental impacts resulting in an increase in ultraviolet radiation.

UNIVERSITY OF MINNESOTA, DULUTH, MINNESOTA (NO1-ES-4-2149)

TITLE: "Collection and Storage of Biologic Specimens Exposed to Orally Ingested Asbestos Fibers"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Carter, M.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D., Research Contracts Office, RRP

DATE CONTRACT INITIATED: November 5, 1973

CURRENT ANNUAL LEVEL: $13,000

PROJECT DESCRIPTION

OBJECTIVES: Collection and maintenance of selected tissue samples relevant to the epidemiology of possible human health effects of asbestos ingestion.

METHODS EMPLOYED: Collected specimens will be identified and preserved to assure usefulness for current and future analyses.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor has been successful in collection and storage of specimens as proposed. The contractor will continue to provide for long-term retention of collected specimens.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will produce asbestos tissue burden information basic to determination of the health hazard potential of such exposure. Such studies are an integral part of the NIEHS efforts to determine the magnitude and significance of hazards inherent in long-term, low-level exposures to various environmental agents.

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